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# United States Patent [19]

Kubota et al.

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[54] **DNA ENCODING ENZYME, RECOMBINANT DNA AND ENZYME, TRANSFORMANT, AND THEIR PREPARATIONS AND USES**

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[\*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,716,813.

[21] Appl. No.: **961,240**

[22] Filed: **Oct. 30, 1997**

## Related U.S. Application Data

[60] Continuation of Ser. No. 607,321, Feb. 26, 1996, Pat. No. 5,716,813, which is a division of Ser. No. 399,646, Mar. 7, 1995, Pat. No. 5,556,781.

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Mar. 7, 1994 [JP] Japan ..... 6-59840

[51] **Int. Cl.<sup>6</sup>** ..... C12N 9/24; C12N 9/26;  
C12N 1/02; C13J 1/00

[52] **U.S. Cl.** ..... 435/96; 435/200; 435/201;  
435/276

[58] **Field of Search** ..... 435/96, 200, 201,  
435/276

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## ABSTRACT

Disclosed are a DNA encoding an enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, recombinant DNA and enzyme, transformant, and their preparations and uses. These facilitate the industrial-scale production of trehalose with a relative easiness and low cost, and trehalose thus obtained can be satisfactorily used in a variety of food products, cosmetics and pharmaceuticals.

**7 Claims, 9 Drawing Sheets**

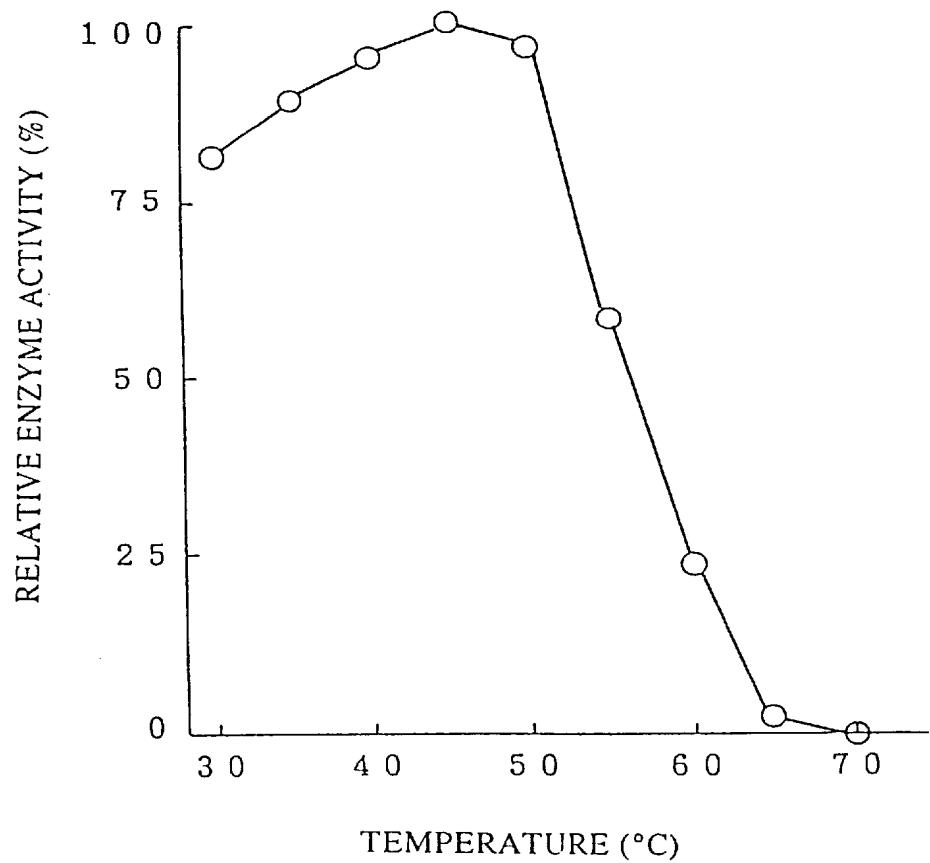


FIG. 1

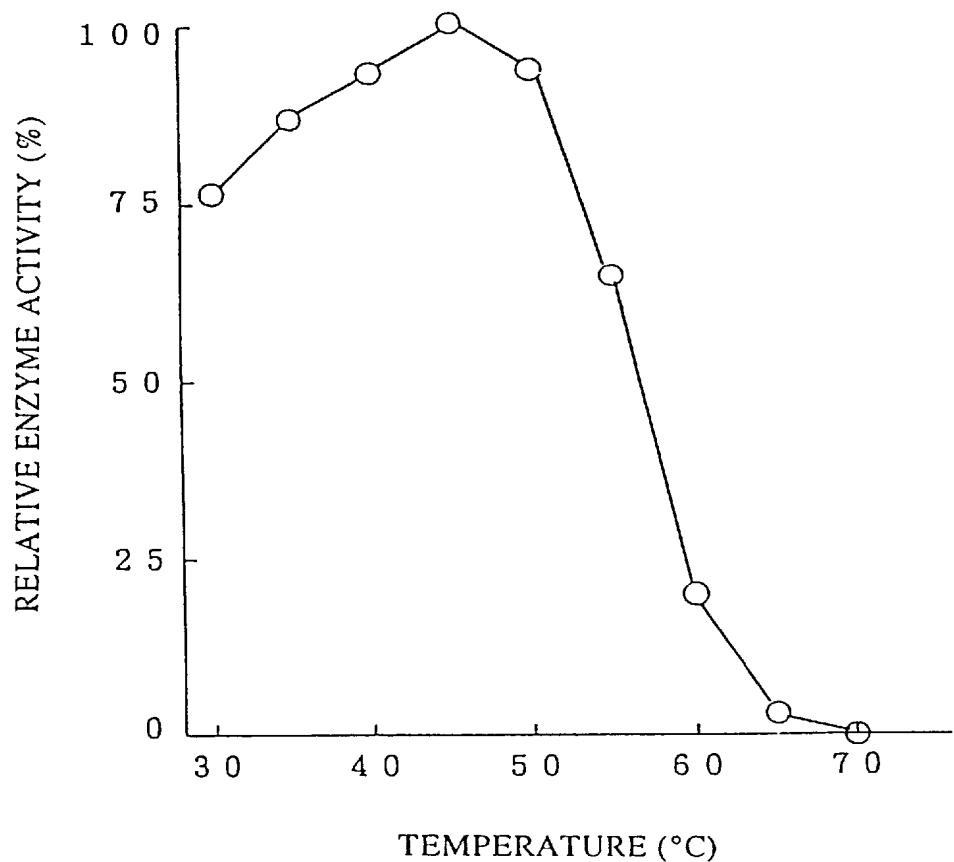


FIG. 2

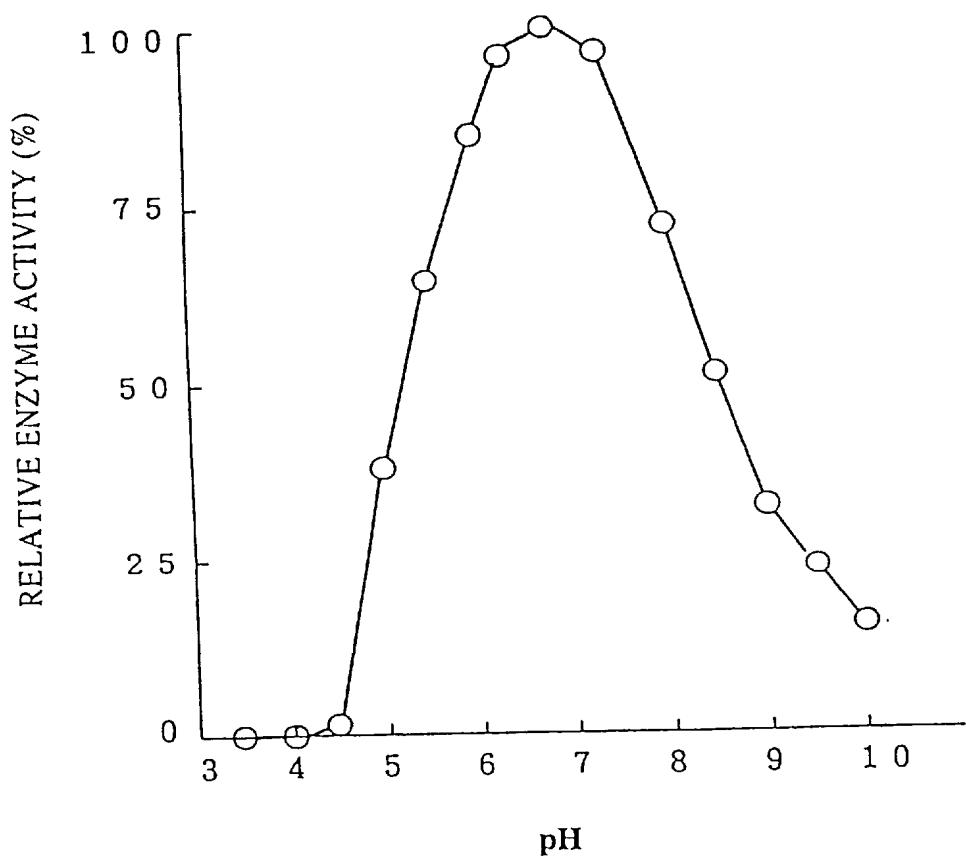


FIG. 3

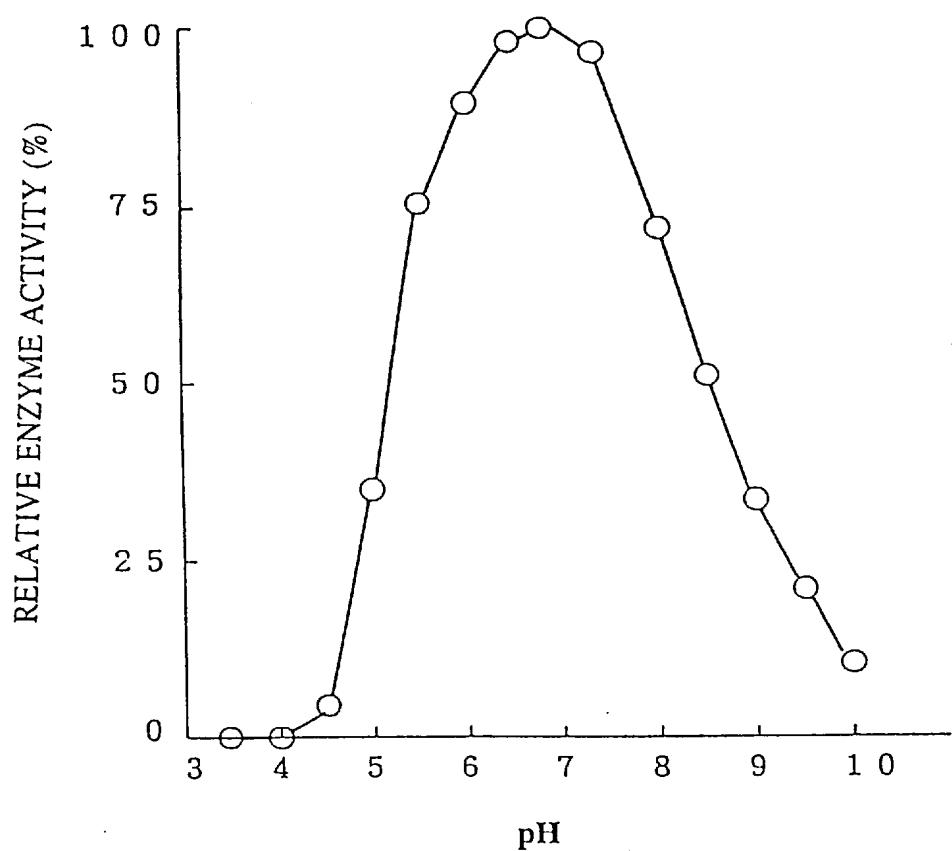


FIG. 4

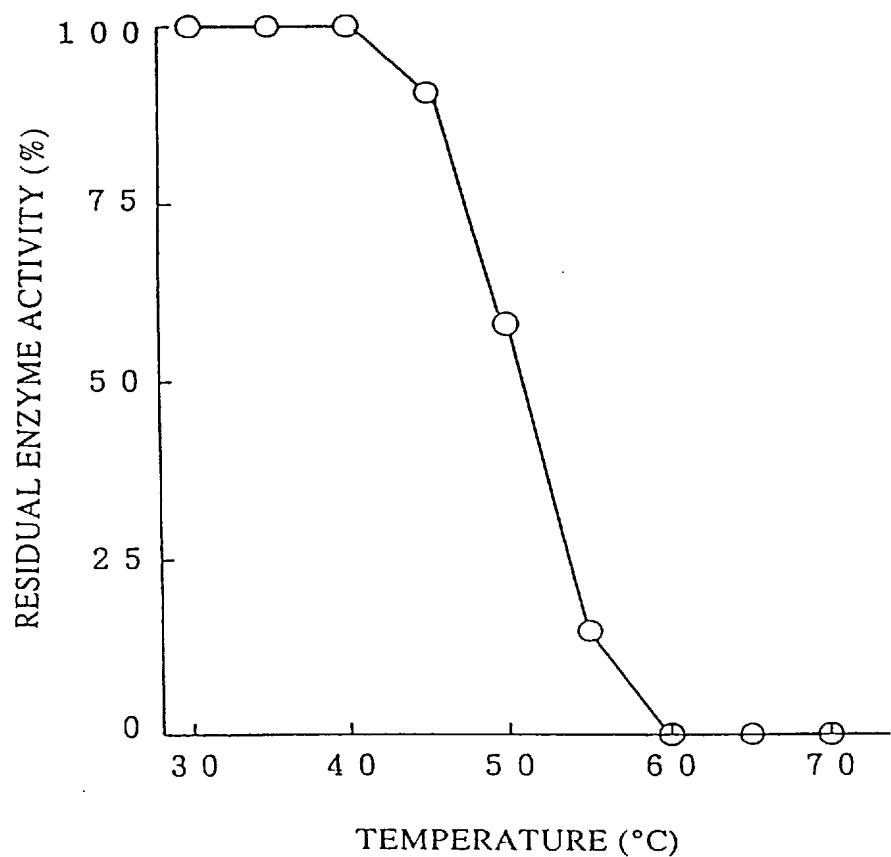


FIG. 5

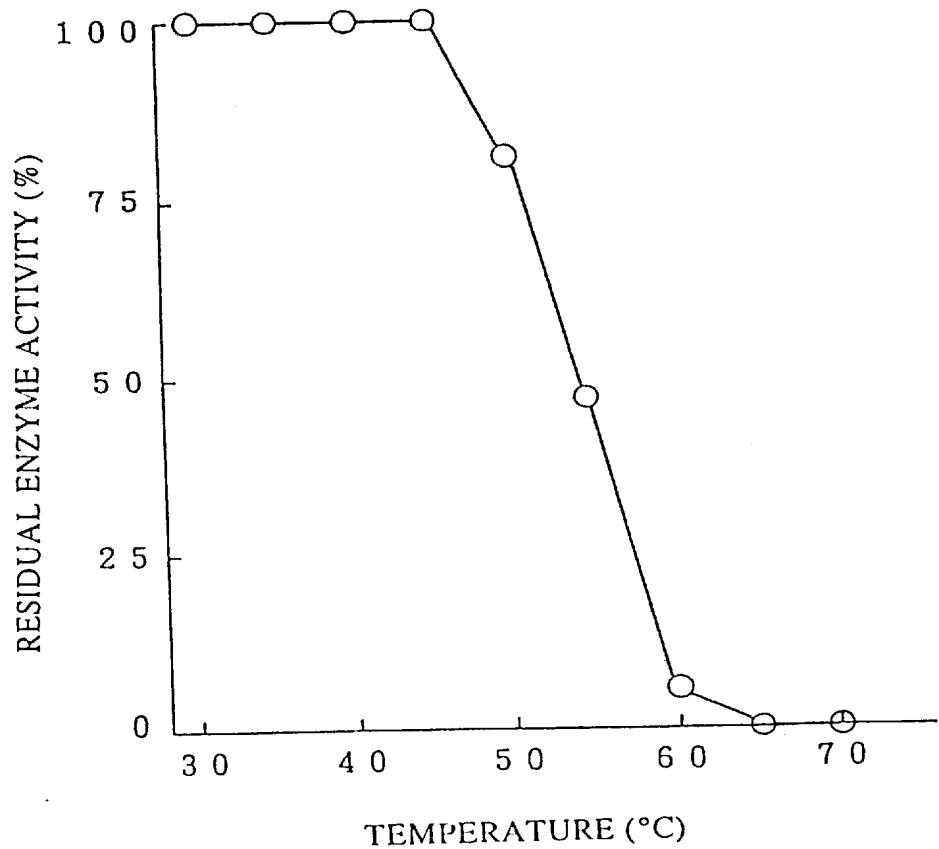
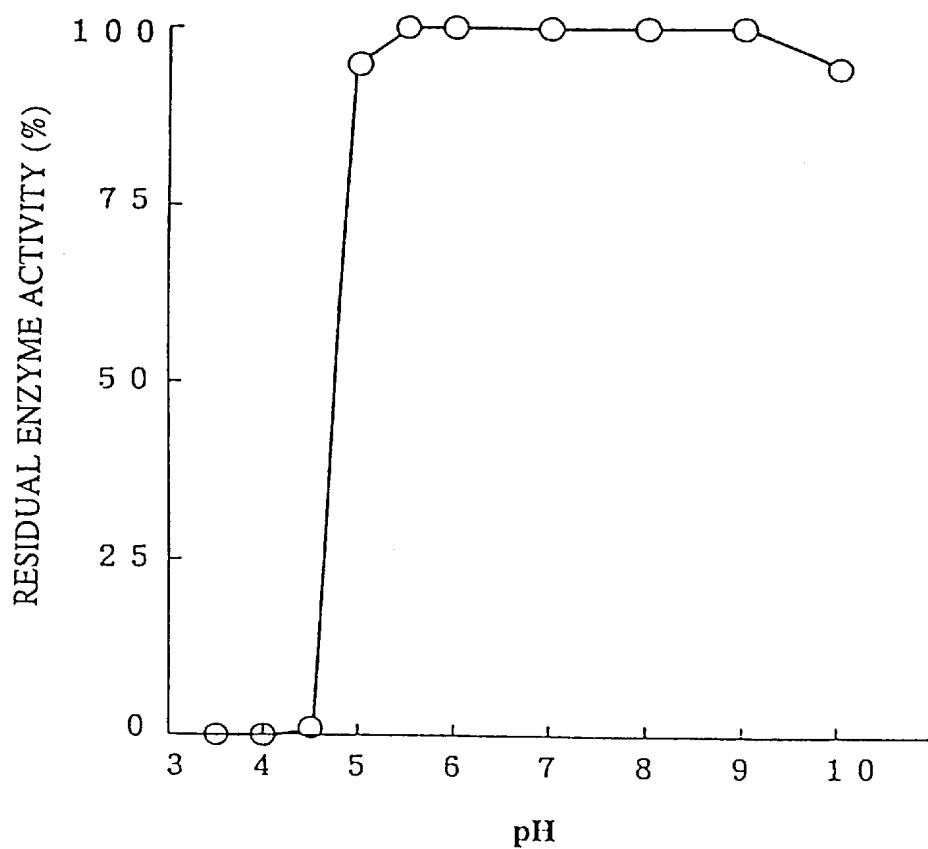
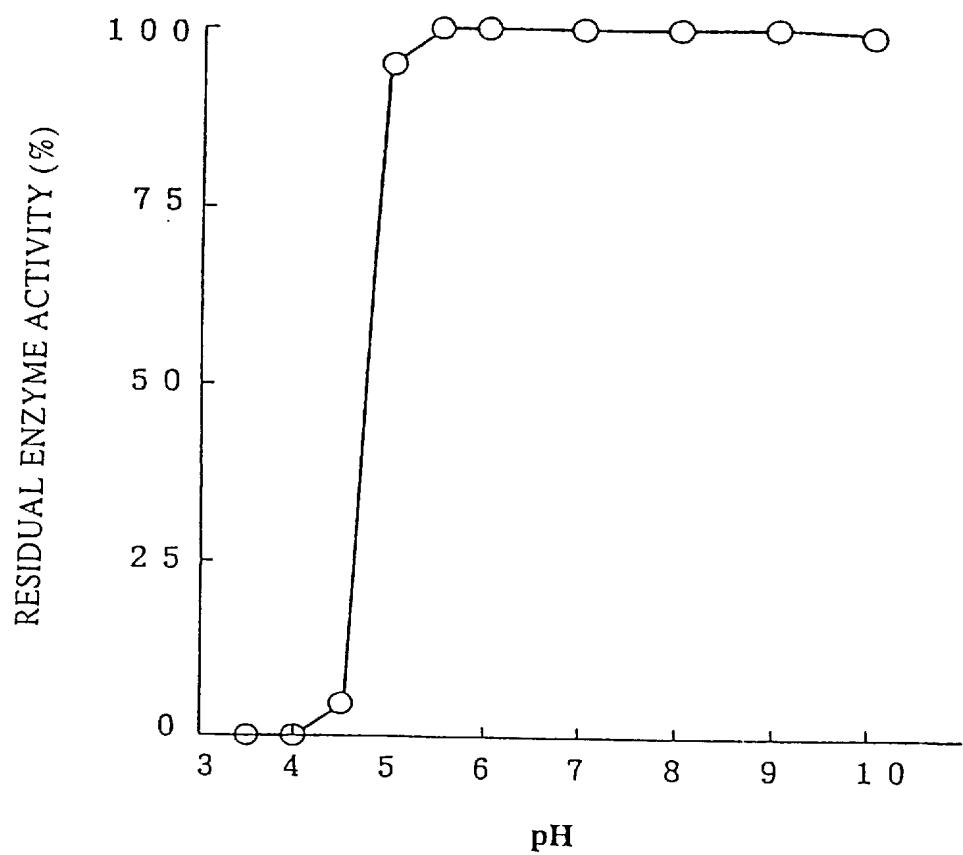


FIG. 6



**FIG. 7**



**FIG. 8**

FIG. 9

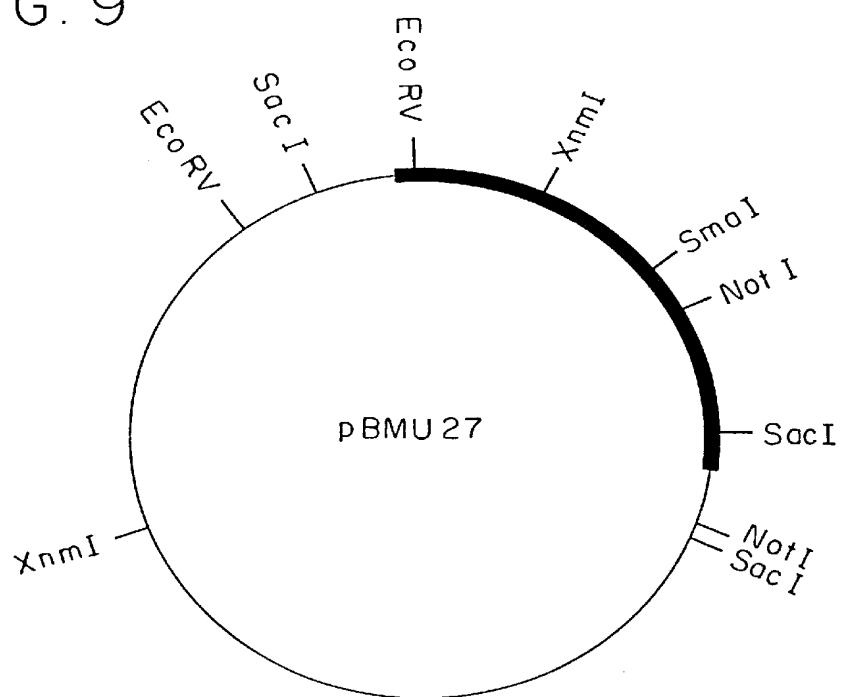
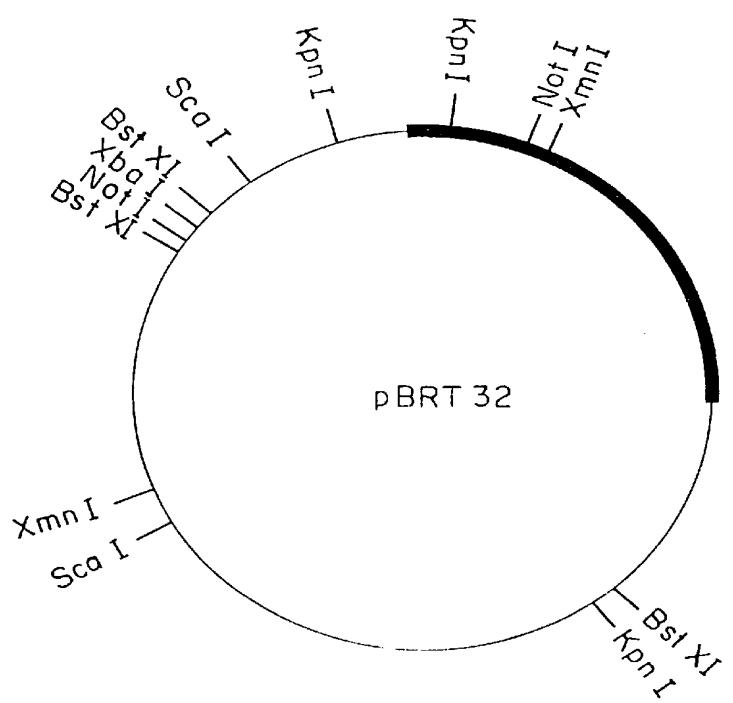


FIG. 10



**DNA ENCODING ENZYME, RECOMBINANT DNA AND ENZYME, TRANSFORMANT, AND THEIR PREPARATIONS AND USES**

This is a continuation of parent application Ser. No. 08/607,321 filed Feb. 26, 1996, now issued as U.S. Pat. No. 5,716,813, which is a divisional of application Ser. No. 08/399,646, filed Mar. 7, 1995, now issued as U.S. Pat. No. 5,556,781.

**BACKGROUND OF THE INVENTION**

**1. Field Of The Invention**

The present invention relates to a novel DNA encoding an enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, recombinant DNA containing the same, and a transformant, and further relates to a recombinant enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, as well as to preparations and uses thereof.

**2. Description Of The Prior Art**

Trehalose is a disaccharide which consists of 2 glucose molecules which are linked together with their reducing groups, and, naturally, it is present in bacteria, fungi, algae, insects, etc., in an extremely small quantity. Having no reducing residue within the molecule, trehalose does not cause an unsatisfactory browning reaction even when heated in the presence of amino acids or the like, and because of this it can sweeten food products without fear of causing unsatisfactory coloration and deterioration. Trehalose, however, is far from being readily prepared in a desired amount by conventional methods, and, actually, it has not scarcely been used for sweetening food products.

Conventional methods are roughly classified into 2 groups, i.e. the one using cells of microorganisms and the other employing a multi-enzymatic system wherein enzymes are allowed to act on saccharides. The former, as disclosed in Japanese Patent Laid-Open No.154,485/75, is a method which comprises allowing to grow microorganisms such as bacteria and yeasts in a nutrient culture medium, and collecting trehalose from the proliferated cells in the resultant culture. The latter, as disclosed in Japanese Patent Laid-Open No.216,695/83, is a method which comprises providing maltose as a substrate, allowing a multi-enzymatic system using maltose- and trehalose-phosphorylases to act on maltose, and isolating the formed trehalose from the reaction system. Although the former facilitates the growth of microorganisms with a relative easiness, it requires a sequentially-complicated step for collecting trehalose from the microorganisms which contain at most 15 w/w % trehalose, on a dry solid basis (d.s.b.). While the latter enables the separation of trehalose itself with a relative easiness, but it is theoretically difficult to increase the trehalose yield by allowing enzymes to act on substrates at a considerably-high concentration because the enzymatic reaction per se is an equilibrium reaction of 2 different types of enzymes and the equilibrium point constantly inclines to the side of forming glucose phosphate.

In view of the foregoing, the present inventors energetically screened enzymes which form saccharides having a trehalose structure from amylaceous saccharides, and found that microorganisms such as those of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36 produce an absolutely novel enzyme which forms non-reducing saccharides having

a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of 3 or higher. Before or after this finding, it was revealed that such non-reducing saccharides are almost quantitatively hydrolyzed into trehalose and glucose and/or maltooligosaccharides by other enzymes produced from the same microorganisms of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36. Since the combination use of such enzymes enables to form a desired amount of trehalose with a relative easiness, the aforementioned objects relating to trehalose would be completely overcome. Insufficient producibility of such enzymes by the microorganisms results in a drawback that a relatively-large scale culture of the microorganisms is inevitable to industrially produce trehalose and/or non-reducing saccharides having a trehalose structure as an end unit.

Recombinant DNA technology has made a remarkable progress in recent years. At present, even an enzyme, whose total amino acid sequence has not yet been revealed, can be readily prepared in a desired amount, if a gene encoding the enzyme was once isolated and the base sequence was decoded, by preparing a recombinant DNA containing a DNA which encodes the enzyme, introducing the recombinant DNA into microorganisms or cells of plants or animals, and culturing the resultant transformants. Under these circumstances, urgently required are the finding of genes which encode these enzymes and the elucidation of their base sequences.

**SUMMARY OF THE INVENTION**

It is an object of the present invention to provide a DNA which encodes an enzyme that releases trehalose from non-reducing saccharides having a trehalose structure as an end unit.

It is a further object of the present invention to provide a replicable recombinant DNA containing the aforesaid DNA.

It is yet another object of the present invention to provide a transformant which is prepared by introducing the recombinant DNA into an appropriate host.

It is a further object of the present invention to prepare the aforesaid enzyme by the application of the recombinant DNA technology.

It is a further object of the present invention to provide a preparation of the enzyme.

It is a further object of the present invention to provide a method for converting non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The first object of the present invention is attained by a DNA which encodes an enzyme that releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The second object of the present invention is attained by a replicable recombinant DNA which contains the aforesaid DNA and a self-replicable vector.

The third object of the present invention is attained by a transformant prepared by introducing the aforesaid self-replicable vector into an appropriate host.

The fourth object of the present invention is attained by a recombinant enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The fifth object of the present invention is attained by a process to produce the recombinant enzyme comprising

culturing a transformant capable of forming the enzyme in a nutrient culture medium, and recovering the formed enzyme from the resultant culture.

The sixth object of the present invention is attained by a method for converting non-reducing saccharides containing a step of allowing the recombinant enzyme to act on non-reducing saccharides, having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, to release trehalose from the saccharides.

#### BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

FIG. 1 shows the optimum temperature of an enzyme derived from *Rhizobium* sp. M-11.

FIG. 2 shows the optimum temperature of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. 3 shows the optimum pH of an enzyme derived from *Rhizobium* sp. M-11.

FIG. 4 shows the optimum pH of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. 5 shows the thermal stability of an enzyme derived from *Rhizobium* sp. M-11.

FIG. 6 shows the thermal stability of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. 7 shows the pH stability of an enzyme derived from *Rhizobium* sp. M-11.

FIG. 8 shows the pH stability of an enzyme derived from *Arthrobacter* sp. Q36.

FIG. 9 shows the restriction map of the recombinant DNA pBMU27 according to the present invention. In the figure, the bold-lined part is a DNA encoding an enzyme derived from *Rhizobium* sp. M-11.

FIG. 10 shows the restriction map of the recombinant DNA pBRT32 according to the present invention. In the figure, the bold-lined part is a DNA encoding an enzyme derived from *Arthrobacter* sp. Q36.

#### DETAILED DESCRIPTION OF THE INVENTION

The DNA according to the present invention exerts the production of the enzyme encoded by the DNA in a manner that the DNA is inserted into an appropriate self-replicable vector to form a replicable recombinant DNA, followed by introducing the recombinant DNA into a host, incapable of producing the enzyme per se but readily replicable, to form a transformant.

Although the recombinant DNA per se does not produce the enzyme, the production of the enzyme encoded by the DNA is attained by introducing the recombinant DNA into a host, incapable of producing the enzyme but replicable with a relative easiness, to form a transformant, and culturing the transformant to produce the enzyme.

The transformant according to the present invention produces the enzyme when cultured.

The recombinant enzyme according to the present invention releases trehalose when acts on non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

The recombinant enzyme is readily obtained in a desired amount by culturing the transformant according to the invention.

Non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization

of 3 or higher are converted into trehalose and glucose and/or maltooligosaccharides.

The present invention is based on the finding of a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Such an enzyme can be obtained from cultures of microorganisms of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36, and the present inventors isolated the enzyme by the combination use of conventional purification methods using column chromatography mainly, examined the properties and features, and revealed the reality, i.e. it is a polypeptide having the following physicochemical properties:

- (1) Action Releasing trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher;
- (2) Molecular weight About 57,000–68,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE);
- (3) Isoelectric point About 3.3–4.6 on isoelectrophoresis;
- (4) Optimum temperature Exhibiting an optimum temperature of around 35°–45° C. when incubated at pH 7.0 for 30 min;
- (5) Optimum pH Exhibiting an optimum pH of around 6.0–7.5 when incubated at 40° C. for 30 min;
- (6) Thermal stability Stable up to a temperature of around 30°–45° C. when incubated at pH 7.0 for 60 min; and
- (7) pH Stability Stable up to a pH of around 5.5–10.0 when incubated at 25° C. for 16 hours.

Experiments, which were conducted to reveal the physicochemical properties of the enzymes produced by microorganisms of the species *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36 (the enzymes from *Rhizobium* sp. M-11 and *Arthrobacter* sp. Q36 are respectively designated as “enzyme M-11” and “enzyme Q36” hereinafter), are explained in the below:

##### Experiment 1

###### Purification of enzyme

###### Experiment 1-1

###### Purification of enzyme M-11

In 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid culture medium (pH 7.0) containing 2.0 w/v % “PINE-DEX #4”, a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, Japan, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate, and 0.1 w/v % potassium dihydrogen phosphate, and the flasks were autoclaved at 120° C. for 20 min to effect sterilization. After cooling the flasks a seed culture of *Rhizobium* sp. M-11 was inoculated into each liquid culture medium in each flask, followed by the incubation at 27° C. for 24 hours under rotary-shaking conditions. Twenty L of a fresh preparation of the same liquid culture medium was put in a 30-L jar fermentor and sterilized, followed by inoculating one v/v % of the culture obtained in the above into the sterilized liquid culture medium in the jar fermentor, and incubating it at a pH of 6–8 and 30° C. for 24 hours under aeration-agitation conditions.

Thereafter, about 18 L of the resultant culture was subjected to an ultra-high pressure cell disrupting apparatus to disrupt cells. The resultant suspension was centrifuged to obtain a supernatant, and to about 16 L of which was added ammonium sulfate to give a 20 w/v % saturation, followed

by the standing at 4° C. for one hour and the centrifugation to remove sediment. To the resultant supernatant was added ammonium sulfate to give a 60 w/v % saturation, and the

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solution was allowed to stand at 4° C. for 24 hours and centrifuged to collect sediment which was then dissolved in a minimum amount of 10 mM phosphate buffer (pH 7.0). The solution thus obtained was dialyzed against 10 mM phosphate buffer (pH 7.0) for 24 hours, and centrifuged to remove insoluble substances. The resultant supernatant was fed to a column packed with "DEAE-TOYOPEARL®", a product for ion-exchange chromatography commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0), followed by feeding to the column a linear gradient buffer of sodium chloride ranging from 0 M to 0.5 M in 10 mM phosphate buffer (pH 7.0). Fractions containing the objective enzyme were collected from the eluate, pooled, dialyzed for 10 hours against 50 mM phosphate buffer (pH 7.0) containing 2 M ammonium sulfate, and centrifuged to remove insoluble substances. Thereafter, the resultant supernatant was fed to a column, which had been packed with "BUTYL TOYOPEARL®", a gel for hydrophobic column chromatography commercialized by Tosoh Corporation, Tokyo, Japan, and equilibrated with 50 mM phosphate buffer (pH 7.0) containing 2 M ammonium sulfate, followed by feeding to the column a linear gradient buffer of ammonium sulfate ranging from 2 M to 0 mM in 50 mM phosphate buffer (pH 7.0). Fractions containing the objective enzyme were collected from the eluate, pooled, fed to a column packed with "TOYOPEARL® HW-55", a product for gel filtration column chromatography commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 50 mM phosphate buffer (pH 7.0), followed by feeding to the column 50 mM phosphate buffer (pH 7.0) and collecting fractions containing the objective enzyme. The enzyme thus obtained had a specific activity of about 240 units/mg protein, and the yield was about 650 units per L of the culture.

Throughout the specification the enzyme activity is expressed by the value measured on the following assay: Place 4 ml of 50 mM phosphate buffer (pH 7.0) containing 1.25 w/v % maltotriosyltrehalose in a test tube, add one ml of an enzyme solution to the tube, and incubate the resultant solution at 40° C. for 30 min to effect enzymatic reaction. Thereafter, one ml of the reaction mixture is mixed with 2 ml of copper reagent to suspend the enzymatic reaction, followed by assaying the reducing activity by the Somogyi-Nelson's method. As a control, an enzyme, which has been previously inactivated by heating at 100° C. for 10 min, is similarly treated as above. One unit activity of the enzyme is defined as the amount of enzyme which increases the reducing power corresponding to one  $\mu$ mol glucose per min under the above conditions.

## Experiment 1-2

## Purification of enzyme Q36

Similarly as in Experiment 1-1, a seed culture of *Arthrobacter* sp. Q36 was cultured, and the resultant culture was treated to obtain a purified enzyme Q36 having a specific activity of about 450 units/mg protein in a yield of about 650 units per L of the culture.

## Experiment 2

## Physicochemical property of enzyme

## Experiment 2-1

## Action

According to the method disclosed in Japanese Patent Application No.349,216/93, a non-reducing saccharide containing 98 w/w % or higher, d.s.b.,  $\alpha$ -glucosyltrehalose,  $\alpha$ -maltosyltrehalose,  $\alpha$ -maltotriosyltrehalose,  $\alpha$ -maltotetraosyltrehalose or  $\alpha$ -malopentaosyltrehalose. Either of the non-reducing saccharides as a substrate was

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dissolved in 50 mM phosphate buffer (pH 7.0) into a 20 w/v % solution which was then mixed with 2 units/g substrate of the purified enzyme M-11. or Q36 in Experiment 1 and subjected to an enzymatic reaction at 40° C. for 48 hours. The reaction mixture was desalted in usual manner, fed to "WB-T-330", a column for high-performance liquid chromatography (HPLC) commercialized by Wako Pure Chemical Industries, Ltd., Tokyo, Japan, followed by feeding to the column distilled water at a flow rate of 0.5 ml/min at ambient temperature to isolate saccharides contained in the reaction mixture while monitoring the saccharide concentration of the eluate with "MODEL RI-8012", a differential refractometer commercialized by Tosoh Corporation, Tokyo, Japan. As a control, an aqueous solution which contains either maltotriose, maltotetraose, malopentaose, malohexaose or maloheptaose was similarly treated as above, and the resultant mixture was analyzed. The saccharide compositions of the reaction mixtures were tabulated in Tables 1 and 2.

TABLE 1

| Substrate                         | Saccharide in reaction mixture | Saccharide composition (%) |
|-----------------------------------|--------------------------------|----------------------------|
| $\alpha$ -Glucosyltrehalose       | Trehalose                      | 17.5                       |
|                                   | Glucose                        | 6.5                        |
| $\alpha$ -Maltosyltrehalose       | Trehalose                      | 44.3                       |
|                                   | Maltose                        | 44.4                       |
| $\alpha$ -Maltotriosyltrehalose   | Trehalose                      | 39.5                       |
|                                   | Maltotriose                    | 60.0                       |
| $\alpha$ -Maltotetraosyltrehalose | Trehalose                      | 0.5                        |
|                                   | Maltotetraose                  | 34.2                       |
| $\alpha$ -Malopentaosyltrehalose  | Trehalose                      | 65.5                       |
|                                   | Malopentaose                   | 0.3                        |
| Maltotriose                       | Trehalose                      | 29.1                       |
|                                   | Malopentaose                   | 70.6                       |
| Maltotetraose                     | Trehalose                      | 0.3                        |
|                                   | Maltotetraose                  | 100.0                      |
| Malopentaose                      | Trehalose                      | 100.0                      |
|                                   | Malopentaose                   | 100.0                      |
| Malohexaose                       | Trehalose                      | 100.0                      |
|                                   | Malohexaose                    | 100.0                      |
| Maloheptaose                      | Trehalose                      | 100.0                      |
|                                   | Maloheptaose                   | 100.0                      |

TABLE 2

| Substrate                         | Saccharide in reaction mixture | Saccharide composition (%) |
|-----------------------------------|--------------------------------|----------------------------|
| $\alpha$ -Glucosyltrehalose       | Trehalose                      | 19.3                       |
|                                   | Glucose                        | 10.2                       |
| $\alpha$ -Maltosyltrehalose       | Trehalose                      | 70.5                       |
|                                   | Maltose                        | 44.5                       |
| $\alpha$ -Maltotriosyltrehalose   | Trehalose                      | 44.4                       |
|                                   | $\alpha$ -Maltosyltrehalose    | 11.1                       |
| $\alpha$ -Maltotetraosyltrehalose | Trehalose                      | 38.8                       |
|                                   | Maltotriose                    | 60.7                       |
| $\alpha$ -Malopentaosyltrehalose  | Trehalose                      | 0.5                        |
|                                   | Maltotetraose                  | 34.1                       |
| Maltotriose                       | Trehalose                      | 65.7                       |
|                                   | Malopentaose                   | 0.2                        |
| $\alpha$ -Malopentaosyltrehalose  | Trehalose                      | 29.3                       |
|                                   | Malopentaose                   | 70.4                       |
| Maltotetraose                     | Trehalose                      | 0.3                        |
|                                   | Maltotriose                    | 100.0                      |
| Malopentaose                      | Trehalose                      | 100.0                      |
|                                   | Malopentaose                   | 100.0                      |
| Malohexaose                       | Trehalose                      | 100.0                      |
|                                   | Malohexaose                    | 100.0                      |
| Maloheptaose                      | Trehalose                      | 100.0                      |
|                                   | Maloheptaose                   | 100.0                      |

As shown in Tables 1 and 2, enzymes M-11 and Q36 almost quantitatively released trehalose, glucose and malooligosaccharides from non-reducing saccharides having a

trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. These enzymes did not act on maltooligosaccharides, as a substrate, having a degree of glucose polymerization of 3 or higher. These facts indicate that these enzymes selectively act on non-reducing saccharides having a trehalose structure as an end unit and having a degree of polymerization degree of 3 or higher, and specifically hydrolyze the glycosidic bond between trehalose- and glycosyl-residues. Such an enzyme has never been reported and is estimated to have a novel enzymatic reaction mechanism.

#### Experiment 2-2

##### Molecular weight

In accordance with the method reported by U. K. Laemmli in *Nature*, Vol.227, pp.680-685 (1970), the purified enzymes M-11 and Q36 in Experiment 1 were respectively electrophoresed on sodium dodecyl sulfate polyacrylamide gel electrophoresis to show a single protein band at a position corresponding to about 57,000-68,000 daltons. The marker proteins used in this experiment were myosin (MW=200,000 daltons),  $\beta$ -galactosidase (MW=116,250 daltons), phosphorylase B (MW=97,400 daltons), serum albumin (MW=66,200 daltons) and ovalbumin (MW=45,000 daltons).

#### Experiment 2-3

##### Isoelectric point

The purified enzymes M-11 and Q36 obtained in Experiment 1 gave an isoelectric point of about 3.3-4.6 on iso-electrophoresis.

#### Experiment 2-4

##### Optimum temperature

The optimum temperature of the purified enzymes M-11 and Q36 obtained in Experiment 1 was about 35°-45° C. as shown in FIGS. 1 and 2 when incubated in usual manner in 50 mM phosphate buffer (pH 7.0) for 30 min.

#### Experiment 2-5

##### Optimum pH

The optimum pH of the purified enzymes M-11 and Q36 obtained in Experiment 1 was about 6.0-7.5 as shown in FIGS. 3 and 4 when experimented in usual manner by incubating them at 40° C. for 30 min in 50 mM acetate buffer, phosphate buffer or sodium carbonate-sodium hydrogen carbonate buffer having different pHs.

#### Experiment 2-6

##### Thermal stability

The purified enzymes M-11 and Q36 obtained in Experiment 1 were stable up to a temperature of about 30°-45° C. as shown in FIGS. 5 and 6 when experimented in usual manner by incubating them in 50 mM phosphate buffer (pH 7.0) for 60 min.

#### Experiment 2-7

##### pH Stability

The purified enzymes M-11 and Q36 obtained in Experiment 1 were stable up to a pH of about 5.5-10.0 as shown in FIGS. 7 and 8 when experimented in usual manner by incubating them at 25° C. for 16 hours in 50 mM acetate buffer, phosphate buffer or sodium carbonate-sodium hydrogen carbonate buffer having different pHs.

#### Experiment 2-8

##### Amino acid sequence containing the N-terminal

The amino acid sequence containing the N-terminal of the purified enzyme M-11 obtained in Experiment 1 was analyzed on "MODEL 470A", a gas-phase protein sequencer commercialized by Applied Biosystems, Inc., Foster City, USA, to reveal that it has the amino acid sequence as shown in SEQ ID NO: 5.

The amino acid sequence containing the N-terminal of the purified enzyme Q36 was analyzed similarly as above to reveal that it has the amino acid sequence as shown in SEQ ID NO: 6.

#### Experiment 2-9

##### Partial amino acid sequence

An adequate amount of the purified enzyme M-11 obtained in Experiment 1-1 was weighed, dialyzed against 10 mM Tris-HCl buffer (pH 9.0) at 4° C. for 18 hours, and admixed with 10 mM Tris-HCl buffer (pH 9.0) to give a concentration of about one mg/ml of the enzyme. About one ml of the resultant solution was placed in a container, admixed with 10  $\mu$ g lysyl endopeptidase, and incubated at 30° C. for 22 hours to partially hydrolyze the enzyme. The resultant hydrolysate was applied to "CAPCELL-PAK C18", a column for reverse-phase high-performance liquid chromatography commercialized by Shiseido Co., Ltd., Tokyo, Japan, which had been previously equilibrated with 0.1 v/v % trifluoroacetate containing 16 v/v % aqueous acetonitrile, followed by feeding to the column 0.1 v/v % trifluoroacetate at a flow rate of 0.9 ml/min while increasing the concentration of acetonitrile from 16 v/v % to 64 v/v % to separately collect fractions containing a peptide fragment eluted about 43 min or about 57 min after the initiation of feeding (the peptide fragments were respectively named "peptide fragment A" and "peptide fragment B"). Fractions containing the peptide fragment A or B were separately pooled, dried in vacuo, and dissolved in 0.1 v/v % trifluoroacetate containing 50 v/v % aqueous acetonitrile. Similarly as in Experiment 2-8, the peptide fragments A and B were analyzed to reveal that they have the amino acid sequences as shown in SEQ ID NOs: 7 and 8, respectively.

Similarly as in enzyme M-11, enzyme Q36 obtained in Experiment 1-2 was partially hydrolyzed, and the resultant was fed to " $\mu$ BONDAPAK C18", a column for reverse-phase high-performance liquid chromatography commercialized by Japan Millipore Ltd., Tokyo, Japan, which had been previously equilibrated with 0.1 v/v % trifluoroacetate containing 24 v/v % aqueous acetonitrile, followed by feeding to the column 0.1 v/v % trifluoroacetate containing 24 v/v % aqueous acetonitrile while increasing the concentration of aqueous acetonitrile from 24 v/v % to 44 v/v % at a flow rate of 0.9 ml/ml. Fractions containing a peptide fragment eluted about 4 min or about 24 min after the initiation of feeding (the fractions were respectively called "peptide fragment C" and "peptide fragment D" hereinafter) were respectively collected, pooled, dried in vacuo, and dissolved in 0.1 v/v % trifluoroacetate containing 50 v/v % aqueous acetonitrile. Analyses of the peptide fragments C and D conducted similarly as above have revealed that they have amino acid sequences as shown in SEQ ID NOs: 9 10 respectively.

No enzyme having these physicochemical properties has been known, and this concluded that it is a novel substance. Referring to *Rhizobium* sp. M-11, it is a microorganism which was isolated from a soil of Okayama-city, Okayama, Japan, deposited on Dec. 24, 1992, in National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology, Tsukuba, Ibaraki, Japan, and accepted under the accession number of FERM BP-4130, and it has been maintained by the institute. *Arthrobacter* sp. Q36 is a microorganism which was isolated from a soil of Soja-city, Okayama, Japan, deposited on Jun. 3, 1993, in the same institute, and accepted under the accession number of FERM BP-4316, and it has been maintained by the institute. Japanese Patent Application No.340,343/93, applied by the same applicant, discloses the properties and features of the non-reducing saccharide-forming enzyme as well as the detailed bacteriological properties of these microorganisms.

The present inventors energetically screened the chromosomal DNA of *Rhizobium* sp. M-11 by using an oligonucle-

otide as a probe which had been chemically synthesized based on the partial amino acid sequence of enzyme M-11 as revealed in Experiment 2-8 or 2-9, and obtained a DNA fragment which consists of 1,767 base pairs having the base sequence as shown in the following SEQ ID NO: 1 that initiates from the 5'-terminus. The decoding of the base sequence of the enzyme has revealed that it has an amino acid sequence consisting of 589 amino acids as shown in SEQ ID NO: 2.

Similarly as in enzyme M-11, the chromosomal DNA of enzyme Q36 was screened by using an oligonucleotide as a probe which had been chemically synthesized based on a partial amino acid sequence of enzyme Q36, and this yielded a DNA fragment having a base sequence consisting of 1,791 base pairs as shown in SEQ ID NO: 3. The base sequence was decoded to reveal that enzyme Q36 has an amino acid sequence consisting of 597 amino acids as shown in SEQ ID NO: 4.

The sequential experimental steps used for revealing the base sequence and amino acid sequence as shown in SEQ ID NOs: 1 to 4 are summarized as below:

- (1) The enzyme was isolated from a culture of a donor microorganism and highly purified. The purified enzyme was partially hydrolyzed with protease, and the resultant 2 different types of peptide fragments were isolated and determined their amino acid sequences;
- (2) Separately, a chromosomal DNA was isolated from a donor microorganism's cell, purified and partially digested by a restriction enzyme to obtain a DNA fragment consisting of about 2,000–6,000 base pairs. The DNA fragment was ligated by DNA ligase to a plasmid vector, which had been previously cut with a restriction enzyme, to obtain a recombinant DNA;
- (3) The recombinant DNA was introduced into *Escherichia coli* to obtain transformants, and from which an objective transformant containing a DNA encoding the enzyme was selected by the colony hybridization method using an oligonucleotide, as a probe, which had been chemically synthesized based on the aforesaid partial amino acid sequence; and
- (4) The recombinant DNA was obtained from the selected transformant and annealed with a primer, followed by allowing a DNA polymerase to act on the resultant to extend the primer, and determining the base sequence of the resultant complementary chain DNA by the dideoxy chain termination method. The comparison of an amino acid sequence, estimable from the determined base sequence with the aforesaid amino acid sequence, confirmed that the base sequence encodes the enzyme.

The recombinant enzyme as referred to in the specification mean the whole recombinant enzymes which are preparable by the recombinant DNA technology and capable of releasing trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Generally, the recombinant enzyme according to the present invention has a revealed amino acid sequence, and, as an example, the amino acid sequence as shown in SEQ ID NO: 2 or 4 which initiates from the N-terminal, as well as homologous ones to it, can be mentioned. Variants having amino acid sequences homologous to the one as shown in SEQ ID NO: 2 or 4 can be obtained by replacing one or more amino acids in SEQ ID NO: 2 or 4 with other amino acids without substantially altering the inherent activity of the enzyme. Although even when used the same DNA and it also depends on hosts into which the DNA is introduced, as well as on ingredients and components of nutrient culture media used for culturing

transformants, and their cultivation temperature and pH, there may be produced modified enzymes which have amino acid sequences similar to that of SEQ ID NO: 2 or 4, as well as having the enzymatic activity inherent to the enzyme encoded by the DNA but defective one or more amino acids located near to the N-terminal of the amino acid sequence of SEQ ID NO: 2 or 4 and/or having one or more amino acids newly added to the N-terminal by the modification of intracellular enzymes of host s after the DNA expression. In view of the technical background in the art, the enzyme as referred to in the present invention includes those which have the amino acid sequence corresponding to that of SEQ ID NO: 2 or 4, and those which substantially have the amino acid sequence as shown in SEQ ID NO: 2 or 4 except that one or more amino acids in the amino acid sequence are defected, newly added to or replaced with other amino acids, as long as they release trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

In this field, it is known that one or more bases in DNAs can be replaced with other bases by the degeneracy of genetic code without altering the amino acid sequences encoded by the DNAs. Based on this the DNA according to the present invention includes DNAs which contain the base sequence of SEQ ID NO: 1 or 3 and other DNAs, wherein one or more bases are replaced with other bases by degeneracy of genetic code, as long as they encode enzymes having the amino acid sequence as shown in SEQ ID NO: 2 or 4 and homologous variants thereof.

According to the today's recombinant DNA technology, the determination of base sequences from the 5'-termini of DNAs define their complementary base sequences. Therefore, the DNA according to the present invention also includes complementary base sequences corresponding to any one of the aforesaid base sequences. Needless to say, one or more bases in the base sequence, which encodes the enzyme or their variants, can be readily replaced with other bases to allow the DNA to actually express the enzyme production in hosts.

The DNA according to the present invention is as described above, and any DNA derived from natural resources and those artificially synthesized can be used in the present invention as long as they have the aforementioned base sequences. The natural resources of the DNA according to the present invention are, for example, microorganisms of the genera *Rhizobium*, *Arthrobacter*, *Brevibacterium* and *Micrococcus*, i.e. *Rhizobium* sp. M-11 (FERM BP-4130), *Arthrobacter* sp. Q36 (FERM BP-4316), *Brevibacterium helovolum* (ATCC 11822) and *Micrococcus roseus* (ATCC 186) from which genes containing the present DNA can be obtained. These microorganisms can be inoculated in nutrient culture media and cultured for about 1–3 days under aerobic conditions, and the resultant cells were collected from the cultures and subjected to ultrasonication or treated with a cell-wall lysis enzyme such as lysozyme or  $\beta$ -glucanase to extract genes containing the present DNA. In this case, a proteolytic enzyme such as protease can be used along with the cell-wall lysis enzyme, and, in the case of treating the cells with ultrasonication, they may be treated in the presence of a surfactant such as sodium dodecyl sulfate (SDS) or treated with freezing- and thawing-methods. The objective DNA is obtainable by treating the resultant with phenol extraction, alcohol sedimentation, centrifugation, protease treatment and/or ribonuclease treatment used in general in the art.

To artificially synthesize the DNA according to the present invention, it can be chemically synthesized by using

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the base sequence as shown in SEQ ID NO: 1 or 3, or can be obtained in plasmid form by inserting a DNA, which encodes the amino acid sequence as shown in SEQ ID NO: 2 or 4, into an appropriate self-replicable vector to obtain a recombinant DNA, introducing the recombinant DNA into an appropriate host to obtain a transformant, culturing the transformant, separating the proliferated cells from the resultant culture, and collecting plasmids containing the DNA from the cells.

The present invention further relates to replicable recombinant DNAs which express the production of the enzyme according to the invention when introduced into microorganisms as well as plant- and animal-cells which do not produce the enzyme inherently but are readily proliferative. Such a recombinant DNA, which generally contains the aforesaid DNA and a self-replicable vector, can be prepared by conventional method with a relative easiness when the material DNA is in hand. Examples of such a vector are plasmid vectors such as pBR322, pUC18, Bluescript II SK(+), pUB110, pTZ4, pCl94, pHV14, TRP7, TEp7, pBS7, etc.; and phage vectors such as λgt·λC, λgt·λB, φ11, φ1, φ105, etc. Among these plasmid- and phage-vectors, pBR322, pUC18, Bluescript II SK(+), λgt·λC and λgt·λB are satisfactorily used in case that the present DNA should be expressed in *Escherichia coli*, while pUB110, pTZ4, pCl94, φ11 and φ105 are satisfactorily used to express the DNA in microorganisms of the genus *Bacillus*. The plasmid vectors pHV14, TRP7, TEp7 and pBS7 are suitably used when the recombinant DNA is allowed to grow in 2 or more hosts.

The methods used to insert the present DNA into such vectors in the present invention may be conventional ones generally used in this field. A gene containing the present DNA and a self-replicable vector are first digested by a restriction enzyme and/or ultrasonic disintegrator, then the resultant DNA fragments and vector fragments are ligated. To digest DNAs and vectors, restriction enzymes which specifically act on nucleotides, particularly, type II restriction enzymes, more particularly, Sau 3AI, Eco RI, Hind III, Bam HI, Sal I, Xba I, Sac I, Pst I, etc., facilitate the ligation of the DNA fragments and vector fragments. The ligation of the DNA fragments and vector fragments is effected by annealing them first if necessary, then subjected to the action of a DNA ligase *in vivo* or *in vitro*. The recombinant DNA thus obtained is replicable without substantial limitation by introducing it into appropriate hosts, and culturing the resultant transformants.

The recombinant DNA according to the present invention can be introduced into appropriate host microorganisms including *Escherichia coli* and those of the genus *Bacillus* as well as actinomycetes and yeasts. In the case of using *Escherichia coli* as a host, it can be cultured in the presence of the recombinant DNA and calcium ion, while in the case of using the microorganisms of the genus *Bacillus* the competent cell method and the colony hybridization method can be employed. Desired transformants can be cloned by the colony hybridization method or by culturing a variety of transformants in nutrient culture media containing non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, and selecting the objective transformants which release trehalose from the non-reducing saccharides.

The transformants thus obtained extracellularly produce the objective enzyme when cultured in nutrient culture media. Generally, liquid media in general supplemented with carbon sources, nitrogen sources and minerals, and, if necessary, further supplemented with a small amount of

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amino acids and vitamins can be used as the nutrient culture media. Examples of the carbon sources are saccharides such as starch, starch hydrolysate, glucose, fructose and sucrose. Examples of the nitrogen sources are organic- and inorganic-  
5 substances containing nitrogen such as ammonia, ammonium salts, urea, nitrate, peptone, yeast extract, defatted soy bean, corn steep liquor and beef extract. Cultures containing the objective enzyme can be prepared by inoculating the transformants into nutrient culture media, and incubating them at a temperature of 25°–65° C. and a pH of 2–8 for about 1–6 days under aerobic aeration-agitation conditions. Such a culture can be used intact as an enzyme preparation, and, usually, it may be disrupted with ultrasonic disintegrator and/or cell-wall lysis enzymes prior to use, followed by  
10 separating the enzyme from the intact cells and cell debris by filtration and/or centrifugation, and purifying the enzyme. The methods used for purifying the enzyme in the invention include conventional ones in general. From cultures the intact cells and cell debris are eliminated and subjected to one or more methods such as concentration, salting out,  
15 dialysis, separately sedimentation, gel filtration chromatography, ion exchange chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectric point electrophoresis.

**25** As is described above, the enzyme exerts a distinct activity of forming trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher, and such an activity has not yet been found in any conventional enzymes.

**30** Therefore, the use of the enzyme facilitates the preparation of trehalose in a relatively-high yield and efficiency from non-reducing saccharides such as α-glucosyltrehalose, α-maltosyltrehalose, α-maltotriosyltrehalose, α-maltotetraosyltrehalose and α-maltpentaosyltrehalose in  
35 a considerably-high yield. These non-reducing saccharides can be obtained in a satisfactorily-high yield from starch hydrolysates, which are obtained by treating amylaceous substances such as starch, amylose and amylopectin prepared with acids and/or amylases, by using non-reducing saccharide-forming enzyme as disclosed in Japanese Patent Application No.349,216/93. Thus, trehalose, whose industrial preparation has been difficult, can be prepared from starch and amylaceous substances as a material with a relative easiness and in a desired amount when the present enzyme and the non-reducing saccharide-forming enzyme, as disclosed in Japanese Patent Application No.349,216/93, are used in combination.

**40** As described in “*Handbook of Amylases and Related Enzymes*”, 1st edition, edited by The Amylase Research Society of Japan, published by Pergamon Press plc, Oxford, England (1988), α-amylase, maltotetraose-forming amylase, maltpentaose-forming amylase and maltohexaose-forming amylase are especially useful to prepare the reducing amylaceous saccharides used in the invention, and, the use of any one of these amylases readily yields amylaceous saccharide mixtures rich in reducing amylaceous saccharides having a degree of glucose polymerization of 3 or higher in a considerably-high yield. If necessary, the combination use of such an amylase and a  
45 starch debranching enzyme such as pullulanase or isoamylase can increase the yield of the reducing amylaceous saccharides usable as a substrate for the non-reducing saccharide-forming enzyme, i.e. the non-reducing saccharides can be obtained by coexisting the non-reducing saccharide-forming enzyme in an aqueous solution containing as a substrate one or more of the reducing amylaceous saccharides in an amount up to a concentration of 50 w/v %,

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and subjecting the solution to an enzymatic reaction at a temperature of about 40°–55° C. and a pH of about 6–8 until a desired amount of the objective non-reducing saccharides are formed.

Usually, in the present conversion method, the recombinant enzyme according to the present invention is allowed to coexist in the aforesaid aqueous solution containing one or more of the non-reducing amylaceous saccharides, and to enzymatically react with the saccharides while keeping at a prescribed temperature and pH until a desired amount of trehalose is released.

Although the enzymatic reaction proceeds even below a concentration of 0.1 w/v % of a substrate, a higher concentration of 2 w/v %, preferably, 5–50 w/v % of a substrate can be satisfactorily used to apply the present conversion method to an industrial-scale production. The temperature and pH used in the enzymatic reaction are set within the ranges of which do not inactivate the recombinant enzyme and allow the recombinant enzyme to effectively act on substrates, i.e. a temperature up to about 55° C., preferably, a temperature in the range of about 40°–55° C., and a pH of 5–10, preferably, a pH in the range of about 6–8. The amount and reaction time of the present recombinant enzyme are chosen dependently on the enzymatic reaction conditions. The enzymatic reaction effectively converts non-reducing saccharides into saccharide compositions containing trehalose and glucose and/or maltooligosaccharides, and, in the case of using  $\alpha$ -maltotriosyltrehalose as a substrate, the conversion rate reaches to approximately 100%. In the case of simultaneously subjecting starch hydrolysates to the action of either of the above amylases together with the non-reducing saccharide-forming enzyme and the present recombinant enzyme, non-reducing saccharides are formed from the hydrolysates while hydrolyzed into glucose and/or maltooligosaccharides, and because of this saccharide compositions with a relatively-high trehalose content can be effectively obtained in a relatively-high yield.

The reaction products obtained by the present conversion reaction can be used intact, and, usually, they are purified prior to use: Insoluble substances are eliminated from the reaction products by filtration and centrifugation, and the resultant solutions are decolorized with activated charcoal, desalted and purified on ion exchangers, and concentrated into syrupy products. Dependently on their use, the syrupy products are dried in vacuo and spray-dried into solid products. In order to obtain products which substantially consist of non-reducing saccharides, the above mentioned syrupy products are subjected to one or more methods such as chromatography using an ion exchanger, activated charcoal and silica gel to separate saccharides, separately sedimentation using alcohol and/or acetone, membrane filtration, fermentation by yeasts, and removal and decomposition of reducing saccharides by alkalis. The methods to treat a large amount of reaction mixture are, for example, fixed bed- or pseudomoving bed-ion exchange column chromatography as disclosed in Japanese Patent Laid-Open Nos. 23,799/83 and 72,598/83, and such a method enables an effective industrial-scale production of products with a relatively-high trehalose content.

These trehalose and compositions containing the same have a wide applicability to a variety of products which are apt to be readily damaged by the reducibility of saccharide sweeteners: For example, they can be satisfactorily used as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant in food products in general, cosmetics and pharmaceuticals.

The following examples explain the present invention in more detail, and the techniques themselves used in the

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examples are conventional ones in this field, for example, those described by J. Sambrook et al. in "Molecular Cloning A Laboratory Manual", 2nd edition, published by Cold Spring Harbor Laboratory Press (1989).

## 5 Example 1

Preparation of recombinant DNA containing DNA encoding enzyme M-11 and transformant

## Example 1-1

Preparation of chromosomal DNA

10 A seed culture of *Rhizobium* sp. M-11 was inoculated into bacto nutrient broth medium (pH 7.0), and cultured at 27° C. for 24 hours with a rotary shaker. The cells were separated from the resultant culture by centrifugation, suspended in TES buffer (pH 8.0), admixed with 0.05 w/v % lysozyme, 15 and incubated at 37° C. for 30 min. The resultant was freezed at -80° C. for one hour, admixed with TSS buffer (pH 9.0), heated to 60° C., and further admixed with a mixture solution of TES buffer and phenol, and the resultant solution was chilled with ice, followed by centrifugally collecting the precipitated crude chromosomal DNA. To the supernatant was added 2 fold volumes of cold ethanol, and the re-precipitated crude chromosomal DNA was collected, suspended in SSC buffer (pH 7.1), admixed with 7.5  $\mu$ g ribonuclease and 125  $\mu$ g protease, and incubated at 37° C. 20 for one hour. Thereafter, a mixture solution of chloroform and isoamyl alcohol was added to the reaction mixture to extract the objective chromosomal DNA, and admixed with cold ethanol, followed by collecting the formed sediment containing the chromosomal DNA. The purified chromosomal DNA thus obtained was dissolved in SSC buffer (pH 7.1) to give a concentration of about one mg/ml, and the resultant solution was freezed at -80° C.

## Example 1-2

Preparation of recombinant DNA pBMU27 and transformant BMU27

35 About one ml of the purified chromosomal DNA obtained in Example 1-1 was placed in a container, admixed with about 35 units of Sau 3AI, a restriction enzyme, and enzymatically reacted at 37° C. for about 20 min to partially 40 digest the chromosomal DNA, followed by recovering a DNA fragment consisting of about 2,000–6,000 base pairs by means of sucrose density-gradient ultracentrifugation. One  $\mu$ g of Bluescript II SK(+), a plasmid vector, was provided, subjected to the action of Bam HI, a restriction enzyme, to completely digest the plasmid vector, admixed with 10  $\mu$ g of the DNA fragment and 2 units of T4 DNA ligase, and allowed to stand at 4° C. overnight to ligate the DNA fragment to the vector fragment. To the resultant recombinant DNA was added 30  $\mu$ l of "Epicurian Coli® 45 XLI-Blue", competent cell commercialized by Toyobo Co., Ltd., Tokyo, Japan, allowed to stand under ice-chilling conditions for 30 min, heated to 42° C., admixed with SOC 50 broth, and incubated at 37° C. for one hour to introduce the recombinant DNA into *Escherichia coli*.

55 The resultant transformant was inoculated into agar plate (pH 7.0) containing 50  $\mu$ g/ml of 5-bromo-4-chloro-3-indolyl- $\beta$ -galactoside, and cultured at 37° C. for 18 hours, followed by placing a nylon film on the agar plate to fix thereon about 6,000 colonies formed on the agar plate.

60 Based on the amino acid sequence located at positions from 8 to 13 as shown in SEQ ID No: 7, i.e. Phe-Asp-Ile-Trp-Ala-Pro, the base sequence of probe 1 represented by 5'-TTYGAYATHGGGCNCC-3' (SEQ ID NO: 15) was chemically synthesized, labelled with  $^{32}$ P, and hybridized 65 with the colonies of transformants fixed on the nylon film, followed by selecting 14 transformants which exhibited a strong hybridization.

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The objective recombinant DNA was selected in usual manner from the 14 transformants, and, in accordance with the method described by E. M. Southern in *Journal of Molecular Biology*, Vol.98, pp.503-517 (1975), the recombinant DNA was hybridized with probe 2 having the base sequence as shown in SEQ ID NO: 8, which had been chemically synthesized based on the amino acid sequence located at positions from 2 to 6, i.e. Asp-Trp-Ala-Glu-Ala, in SEQ ID NO: 8, followed by selecting a recombinant DNA strongly hybridized with the probe 2. The recombinant DNA and transformant thus selected were respectively named "pBMU27" and "BMU27".

The transformant BMU27 was inoculated into L-broth (pH 7.0) containing 100 µg/ml ampicillin, and cultured at 37° C. for 24 hours by a rotary shaker. After completion of the culture, the resultant cells were collected from the culture by centrifugation, and treated with the alkaline method in general to extracellularly extract a recombinant DNA. The extract was in usual manner purified and analyzed to reveal that the recombinant DNA pBMU27 consists of about 5,700 base pairs and has the structure expressed by the restriction map as shown in FIG. 9. It was found that, as shown in FIG. 9, the DNA which consists of 1,767 base pairs for encoding the enzyme M-11 is positioned in the downstream near to the digested site of Eco RV, a restriction enzyme.

**Example 1-3****Production of enzyme by transformant BMU27**

A liquid nutrient culture medium consisting of 2.0 w/v % "PINE-DEX #4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, Japan, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate was adjusted to pH 7.0, admixed with 50 µg/ml ampicillin, autoclaved at 120° C. for 20 min, cooled and inoculated with a seed culture of transformant BMU27 obtained in Example 1-2, followed by culturing the transformant at 37° C. for 24 hours by a rotary shaker. The resultant culture was treated with ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the enzyme activity to find that one L of the culture yielded about 4,000 units of the enzyme.

As a control, a seed culture of *Escherichia coli* XLI-Blue or *Rhizobium* sp. M-11 was inoculated in the same fresh preparation of the same liquid nutrient culture medium but free of ampicillin, and, in the case of culturing *Rhizobium* sp. M-11, it was cultured and treated similarly as above except that the cultivation temperature was set to 30° C. Assaying the resultant activity, one L culture of *Rhizobium* sp. M-11 yielded about 2,000 units of the enzyme, and the yield was significantly lower than that of transformant BMU27. *Escherichia coli* XLI-Blue used as a host did not form the enzyme.

Thereafter, the enzyme produced by the transformant MBU27 was purified similarly as in Experiment 1-1, and examined on the properties and characters. As a result, it was revealed that it has substantially the same physicochemical properties as enzyme M-11, i.e. it has a molecular weight of about 57,000-68,000 daltons on SDS-PAGE and an isoelectric point of about 3.3-4.6 on isoelectrophoresis. The results indicate that the present enzyme can be prepared by the recombinant DNA technology, and the yield can be significantly increased thereby.

**Example 2****Preparation of complementary chain DNA derived from *Rhizobium* sp. M-11, and determination for its base sequence and amino acid sequence****16**

Two µg of the recombinant DNA pBMU27 obtained in Example 1-2 was provided, admixed with 2 M aqueous sodium hydroxide solution to effect degeneration, and admixed with an adequate amount of cold ethanol, followed by collecting the formed sediment containing a template DNA and drying the sediment in vacuo. To the template DNA were added 50 pmole/ml of a chemically synthesized primer 1 represented by 5'-GTAAAACGACGGCCAGT-3' (SEQ ID NO: 16), 10 µl of 40 mM Tris-HCl buffer (pH 7.5) containing 20 mM magnesium chloride and 20 mM sodium chloride, and the mixture was incubated at 65° C. for 2 min to effect annealing and admixed with 2 µl of an aqueous solution containing dATP, dGTP and dTTP in respective amounts of 7.5 µM, 0.5 µl of [ $\alpha$ -<sup>32</sup>P]dCTP (2 mCi/ml), one µl of 0.1 M dithiothreitol, and 2 µl of 1.5 units/ml T7 DNA polymerase, followed by incubating the resultant mixture at 25° C. for 5 min to extend the primer 1 from the 5'-terminus to the 3'-terminus. Thus, a complementary chain DNA was formed.

20 The reaction product containing the complementary chain DNA was divided into quarters, to each of which 2.5 µl of 50 mM aqueous sodium chloride solution containing 80 µM dNTP and 8 µM ddATP, ddCTP, ddGTP or ddTTP was added, and the resultant mixture was incubated at 37° C. for 25 min, followed by suspending the reaction by the addition of 4 µl of 98 v/v % aqueous formamide solution containing 20 mM EDTA, 0.05 w/v % bromophenol blue, and 0.05 w/v % xylene cyanol. The reaction mixture was heated with a boiling-water bath for 3 min, and a portion of which was 30 placed on a gel containing 6 w/v % polyacrylamide, and electrophoresed by energizing the gel with a constant voltage of about 2,000 volts to separate DNA fragments, followed by fixing the gel in usual manner, drying the gel and subjecting the resultant gel to autoradiography.

35 Analyses of the DNA fragments separated on the radiogram revealed that the complementary chain DNA contains the base sequence consisting of about 2,161 base pairs as shown in SEQ ID NO: 11. An amino acid sequence estimable from the base sequence was as shown in SEQ ID NO: 12 and was compared with the amino acid sequence containing the N-terminal or the partial amino acid sequence of enzyme M-11 as shown in SEQ ID NO: 5, 7 or 8. As a result, it was found that the amino acid sequence containing the N-terminal of SEQ ID NO: 5 corresponds to the amino acid sequence located at positions from 8 to 27 in SEQ ID NO: 12, and the partial amino acid sequence of SEQ ID NO: 7 or 8 corresponds to the amino acid sequence located at positions from 10 to 30 or at positions from 493 to 509 in SEQ ID NO: 12. These results indicate that enzyme M-11 has the 40 amino acid sequence of SEQ ID NO: 2, and it is encoded by the DNA having the base sequence as shown in SEQ ID NO: 1.

**Example 3****Preparation of recombinant DNA, containing DNA derived from *Arthrobacter* sp. Q36, and transformant****Example 3-1****Preparation of chromosomal DNA**

Similarly as in Example 1-1, a chromosomal DNA was isolated from *Arthrobacter* sp. Q36, purified and dissolved 55 in SSC buffer (pH 7.1) to give a concentration of about one mg/ml, and the resultant solution was freezed at -80° C. for storage.

**Example 3-2****Preparation of recombinant DNA pBRT32 and transformant BRT32**

The purified chromosomal DNA obtained in Example 3-1 was partially digested similarly as in Example 1-2, followed

by recovering a DNA fragment consisting of about 2,000–6,000 base pairs by sucrose density gradient ultracentrifugation. The DNA fragment was ligated to a lysate of Bluescript II SK(+) which had been treated with Bam HI, and the resultant recombinant DNA was introduced into *Escherichia coli* XLI-Blue. The transformants thus obtained were cultured similarly as in Example 1-2 on agar plates containing 5-bromo-4-chloro-3-indolyl- $\beta$ -galactoside, and the formed about 5,000 colonies were fixed on a nylon film, while the probe 3 represented by 5'-ATGGGNTGGGAYCCNGC-3' (SEQ ID NO: 17) was chemically synthesized based on the amino acid sequence of Met-Gly-Trp-Asp-Pro-Ala located at positions from 5 to 10 in SEQ ID NO: 9, labelled with  $^{32}$ P, and hybridized with transformant colonies which had been fixed on the nylon film, followed by selecting 10 transformants which strongly hybridized with the probe 3.

Similarly as in Example 1-2, the objective recombinant DNA was selected from 10 transformants, and hybridized with probe 4 represented by 5'-TAYGAYGTNTGGGC-3' (SEQ ID NO: 18) which had been chemically synthesized based on the amino acid sequence of Tyr-Asp-Val-Trp-Ala located at positions from 8 to 12 in SEQ ID NO: 10, followed by selecting a recombinant DNA which strongly hybridized with probe 4. The recombinant DNA and transformant thus selected were respectively named "pBRT32" and "BRT32".

The transformant BRT32 was inoculated into L-broth containing ampicillin, and cultured similarly as in Example 1-2, and the proliferated cells were collected from the resultant culture, and from which a recombinant DNA was extracted, purified and analyzed to reveal that the recombinant DNA pBRT32 consists of about 6,200 base pairs and has the structure of the restriction map as shown in FIG. 10. As shown in FIG. 10, it was revealed that the DNA, which consists of 1,791 base pairs for encoding the DNA of enzyme Q36, is located in the downstream near to the cleavage site of Kpn I.

### Example 3-3

#### Production of enzyme by transformant BRT32

A liquid nutrient culture medium consisting of 2.0 w/v % "PINE-DEX #4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate was adjusted to pH 7.0, admixed with 50  $\mu$ g/ml ampicillin, autoclaved at 120° C. for 20 min, cooled and inoculated with a seed culture of the transformant BRT32 obtained in Example 3-2, followed by culturing the transformant at 37° C. for 24 hours by a rotary shaker. The resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the present enzyme activity to find that one L of the culture yielded about 3,900 units of the enzyme.

As a control, a seed culture of *Escherichia coli* XLI-Blue or *Arthrobacter* sp. Q36 was inoculated into a fresh preparation of the same liquid nutrient culture medium but free of ampicillin, and, in the case of culturing *Arthrobacter* sp. Q36, it was cultured and treated similarly as above except that the cultivation temperature was set to 30° C. Assaying the enzyme activity, one L of the culture of *Arthrobacter* sp. Q36 yielded about 1,800 units of the enzyme, and the yield was significantly lower than that of the transformant BRT32. The *Escherichia coli* XLI-Blue used as a host did not form the enzyme.

Thereafter, the enzyme produced by the transformant BRT32 was purified similarly as in Experiment 1-1, and

examined on the properties and characters to reveal that it has substantially the same physicochemical properties as that of enzyme Q36, i.e. it has a molecular weight of about 57,000–68,000 daltons on SDS-PAGE and an isoelectric point of about 3.3–4.6 on isoelectrophoresis. These results indicate that the enzyme can be prepared by the recombinant DNA technology, and the yield can be significantly increased thereby.

### Example 4

#### Preparation of complementary chain DNA derived from *Arthrobacter* sp. Q36, and determination for its base sequence and amino acid sequence

The recombinant DNA pBRT32 obtained in Example 3-2 was similarly treated as in Example 2 to form a template DNA which was then annealed together with the primer 1, followed by allowing T7 DNA polymerase to act on the resultant to extend the primer 1 from the 5'-terminus to the 3'-terminus to obtain a complementary chain DNA. Similarly as in Example 2, the complementary chain DNA was subjected to the dideoxy chain terminator method to analyze DNA fragments which had been isolated on a radiogram. The result revealed that the complementary chain DNA contained a base sequence consisting of 2,056 base pairs as shown in SEQ ID NO: 13. An amino acid sequence estimable from the base sequence was as shown in SEQ ID NO: 14, and compared with the amino acid sequence containing the N-terminal or the partial amino acid sequence of SEQ ID NO: 6, 9 or 10. As a result, it was found that the amino acid sequence of SEQ ID NO: 6 corresponds to that located at positions from 2 to 21 in SEQ ID NO: 14, and that the partial amino acid sequence in SEQ ID NO: 9 or 10 corresponds to that located at positions from 470 to 489 or at positions from 12–31 in SEQ ID NO: 14. These results indicate that enzyme Q36 has the amino acid sequence of SEQ ID NO: 4, and it is encoded by the DNA having the base sequence as shown in SEQ ID NO: 3.

### Example 5

#### Preparation of recombinant enzyme

In 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid nutrient culture medium (pH 7.0) consisting of 2.0 w/v % "PINE-DEX#4", a starch hydrolysate commercialized by Matsutani Chemical Ind., Co., Ltd., Tokyo, Japan, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate, and to each flask was added 50  $\mu$ g/ml ampicillin and autoclaved at 120° C. for 20 min. Thereafter, the flasks were cooled and inoculated with a seed culture of the transformant BMU27 obtained in Example 1-2, followed by culturing the transformant at 27° C. for 24 hours by a rotary shaker. Apart from this, 18 L of a fresh preparation of the same liquid culture medium was placed in a 30-L jar fermentor, admixed with 50  $\mu$ g/ml ampicillin, sterilized at 120° C. for 20 min, cooled and inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 37° C. for 24 hours while keeping the pH at 6–8 under aeration-agitation conditions. The resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the enzyme activity to reveal that one L of the culture yielded about 3,900 units of the enzyme. The supernatant was purified by the method in Experiment 1-1 to obtain an about 67 ml aqueous solution containing an about 165 units/ml of a recombinant enzyme having a specific activity of about 290 units/mg protein.

**Example 6****Preparation of recombinant enzyme**

Recombinant BRT32 obtained by the method in Experiment 3-2 was cultured similarly as in Example 5, and the resultant culture was treated with an ultrasonic integrator to disrupt cells. The resultant suspension was centrifuged to remove insoluble substances, and the resultant supernatant was assayed for the enzyme activity to have an activity of about 4,000 units per L. The supernatant was purified by the method in Experiment 1-1 to obtain an about 55 ml aqueous solution containing about 200 units/ml of a recombinant enzyme with a specific activity of about 420 units/mg protein.

**Example 7****Conversion of non-reducing saccharide by recombinant enzyme****Example 7-1 (a)****Preparation of non-reducing saccharide-forming enzyme**

To 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid nutrient culture medium (pH 7.0) consisting of 2.0 w/v % maltose, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % disodium hydrogen phosphate and 0.1 w/v % potassium dihydrogen phosphate, and the flasks were autoclaved at 120° C. for 20 min. Thereafter, the flasks were cooled and inoculated with a seed culture of *Rhizobium* sp. M-11, followed by culturing it at 27° C. for 24 hours by a rotary shaker. Apart from this, 20 L of a fresh preparation of the same liquid culture medium was placed in a 30-L jar fermentor, and sterilized, inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 30° C. and at a pH of 7-8 for 24 hours under aeration-agitation conditions. Thereafter, the resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances and purified according to the method in Experiment 1-1 to obtain a non-reducing saccharide-forming enzyme having a specific activity of about 195 units/mg protein in a yield of about 220 units per L of the culture.

Throughout the specification the activity of a non-reducing saccharide-forming enzyme is expressed by the value measured on the following assay: Place 4 ml of 50 mM phosphate buffer (pH 7.0) containing 1.25 w/v % maltopentaose in a test tube, add one ml of an enzyme solution to the test tube, and incubate the solution at 40° C. for 60 min to effect enzymatic reaction. Thereafter, the reaction mixture is heated at 100° C. for 10 min to suspend the enzymatic reaction, followed by diluting it with distilled water by 10 times and assaying the reducing activity by the Somogyi-Nelson's method. One unit activity of the non-reducing saccharide-forming enzyme is defined as the amount of enzyme which decreases the reducing power corresponding to one  $\mu$ mol maltopentaose per min under the above conditions.

**Example 7-1(b)****Preparation of syrupy product containing trehalose**

A potato starch was suspended in water to give a 15 w/w % suspension which was then mixed with 0.1 w/w % calcium carbonate. The mixture was adjusted its pH to 6.0, mixed with 0.2 w/w %, d.s.b., of "TERMAMYL 60L", an  $\alpha$ -amylase specimen commercialized by Novo Nordisk Bio-industri A/S, Copenhagen, Denmark, and enzymatically reacted at 95° C. for 15 min to effect gelatinization and

liquefaction. The liquefied solution was autoclaved at 120° C. for 30 min to inactivate the remaining enzyme, rapidly cooled to 45° C., 1,000 units/g starch, d.s.b., of pullulanase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, 3.4 units/g starch, d.s.b., of the non-reducing saccharide-forming enzyme obtained in Example 7-1(a), and 4.2 units/g starch, d.s.b., of the recombinant enzyme obtained by the method in Example 5, followed the enzymatic reaction for 48 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated to obtain a syrupy product with a concentration of about 60 w/w % in a yield of about 92%, d.s.b.

Analysis of the syrup by the method of Experiment 2-1 revealed that it contained 70.2 w/w % trehalose, 2.4 w/w %  $\alpha$ -glucosyltrehalose, 3.3 w/w %  $\alpha$ -maltosyltrehalose, 0.7 w/w % glucose, 10.1 w/w % maltose, 12.9 w/w % maltotriose, and 0.4 w/w % maltooligosaccharides having a degree of glucose polymerization of 4 or higher. The product, having a mild and moderate sweetness as well as an adequate viscosity and moisture-retaining ability, can be satisfactorily used in food products in general, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

**Example 7-1(c)****Preparation of powdery product containing trehalose**

To 4 jacketed-stainless steel columns, having a diameter of 5.4 cm and a length of 5 m each was packed homogeneity with "XT-1016 (Na<sup>+</sup>-form)", a strong-acid cation exchange resin commercialized by Tokyo Organic Chemical Industries, Ltd., Tokyo, Japan, and the columns were cascaded in series to give a total column length of 20 m. The syrupy product obtained in Example 7-1(b) was fed to the columns at a rate of about 5 v/v % against the resin at an inner column temperature of 55° C., and the columns were fed with 55° C. hot water at an SV (space velocity) 0.3 to fractionate saccharides in the syrupy product. Based on the analysis of the saccharide composition of the eluate, fractions rich in trehalose were collected, pooled, concentrated, dried in vacuo and pulverized to obtain a solid product containing about 97 w/w % trehalose in a yield of about 56% against the starting material, d.s.b.

The product, having a mild sweetness and substantially free of reducibility, can be satisfactorily used in food products in general, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

**Example 7-1(d)****Preparation of powdery crystalline trehalose**

A portion of the trehalose rich fraction obtained in Example 7-1(c) was concentrated into an about 75 w/w % solution which was then transferred to a crystallizer, admixed with about 2 w/w %, d.s.b., hydrous crystalline trehalose as a seed crystal, and crystallized under gentle stirring conditions to obtain a massecuite with a crystallinity of about 45 w/w %. The massecuite was sprayed downward from a nozzle, equipped at the upper part of a spraying tower at a pressure of about 150 kg/cm<sup>2</sup> while about 85° C. hot air was flowing downward from the upper part of the tower to

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accumulate a crystalline powder on a belt conveyer provided on the basement of the tower, followed by gradually transferring it out of the tower. Thereafter, the powder was transferred to an ageing tower and aged for 10 hours to complete the crystallization and drying while an about 40° C. hot air was blowing to the contents. Thus, a powdery product containing hydrous crystalline trehalose was obtained in a yield of about 90 w/w % against the starting material, d.s.b.

The product, having a substantial non-hygroscopicity and a mild and high-quality sweetness, can be satisfactorily used in food products in general, cosmetics, pharmaceuticals and feeds as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

**Example 8**

Conversion of non-reducing saccharide by recombinant enzyme

Potato starch was suspended in water to give a concentration of 6 w/w %, d.s.b., and the suspension was admixed with 500 units/g starch of isoamylase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, and enzymatically reacted for 20 hours. The reaction mixture was adjusted to a pH of 6.5, autoclaved at 120° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 95° C., admixed with 0.1 w/w % per g starch, d.s.b., of "TERMAMYL 60L", an  $\alpha$ -amylase specimen commercialized by Novo Nordisk Bioindustri A/S, Copenhagen, Denmark, and enzymatically reacted for 15 min. The reaction mixture was heated at 130° C. for 30 min to inactivate the remaining enzyme, rapidly cooled to 45° C., admixed with 4.1 units/g starch, d.s.b., of a non-reducing saccharide-forming enzyme obtained by the method in Example 7-1(a), and 4.9 units/g starch, d.s.b., of the present recombinant enzyme obtained by the method in Example 6, and enzymatically reacted for 64 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 55° C., adjusted to pH 5.0, admixed with 10 units/g starch, d.s.b., of "GLUCOZYME", a glucoamylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and enzymatically reacted for 40 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated to obtain an about 60 w/w % syrupy product containing about 80.5 w/w % trehalose, d.s.b. The syrupy product was concentrated into an about 84 w/w % syrup which was then transferred to a crystallizer, admixed with an about 2 w/w % hydrous crystalline trehalose, d.s.b., and crystallized under gentle stirring conditions to obtain a massecuite having a crystallinity of about 45 w/w %. The massecuite was distributed to plastic plain vessels which were then allowed to stand at ambient temperature for 3 days to effect solidification and aging, followed by detaching the resultant blocks from the vessels and pulverizing the blocks with a cutter to obtain a solid product containing hydrous crystalline trehalose in a yield of about 90 w/w % against the material starch, d.s.b.

The product, which is substantially free of hygroscopicity and readily handleable, can be arbitrarily used in food products in general, cosmetics, pharmaceuticals as a sweet-

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ening agent, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

**Example 9**

Conversion of non-reducing saccharide by recombinant enzyme

Potato starch was suspended in water to give a concentration of 6 w/w %, d.s.b., and the suspension was admixed with 0.01 w/w % "NEO-SPITASE",  $\alpha$ -amylase commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, 10 adjusted to pH 6.2, and enzymatically reacted at 85°–90° C. for 20 min to gelatinize and liquefy the starch. The liquefied starch was heated at 120° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 45° C., admixed with 500 units/g starch, d.s.b., of isoamylase commercialized by 15 Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, 3.2 units/g starch, d.s.b., of a non-reducing saccharide-forming enzyme obtained by the method in Example 7-1(a), and 5.0 units/g starch, d.s.b., of the present recombinant enzyme obtained by the method in Example 5, and enzymatically reacted for 48 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, rapidly cooled to 55° C., adjusted to pH 5.0, admixed with 10 units/g starch, d.s.b., of 20 "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals Ltd., Kyoto, Japan, and enzymatically reacted for 40 hours. The reaction mixture was heated at 95° C. for 10 min to inactivate the remaining enzyme, rapidly cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated to give a concentration of 25 about 60 w/w %, d.s.b., to obtain a syrupy product containing 78.3 w/w % trehalose, d.s.b. The syrupy product was fractionated similarly as in Example 7-1(c) except for using "CG6000(Na<sup>+</sup>)", a strong-acid cation exchange resin commercialized by Japan Organo, Co., Ltd., Tokyo, Japan, to obtain a fraction containing about 95 w/w % trehalose, d.s.b. The fraction was concentrated to give a concentration of 30 about 75 w/w %, d.s.b., and, similarly as in Example 8, crystallized, and the resultant massecuite in the form of block was pulverized to obtain a powdery product containing hydrous crystalline trehalose in a yield of about 70 w/w % against the material starch, d.s.b.

The product, which is substantially free of hygroscopicity and readily handleable, can be arbitrarily used in food products in general, cosmetics, pharmaceuticals as a sweetening agent, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

As is described above, the present invention is based on the finding that a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. The present invention is to explore a way to produce the enzyme in a relatively-large scale and in a considerably-high yield. The enzyme produced by the transformant according to the present invention is the one characterized by its revealed total amino acid sequence, and because of this it can be used for the preparations of trehalose which is premised on being used in food products without fear of causing side effects.

Therefore, the present invention is an useful invention which exerts the aforesaid significant action and effect as well as giving a great contribution to this field.

## SEQUENCE LISTING

( 1 ) GENERAL INFORMATION:

( i i i ) NUMBER OF SEQUENCES: 18

( 2 ) INFORMATION FOR SEQ ID NO:1:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 1767 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( i x ) FEATURE:

- ( A ) NAME/KEY: CDS
- ( B ) LOCATION: 1..1767

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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| GCC | AAG   | CCG | GTG   | CAG | GGA   | GCG   | GGG   | CAC | TTC   | GAT   | ATC   | TGG   | GCG   | CCC | GAG | 4 8   |  |
| Ala | Lys   | Pro | Val   | Gln | Gly   | Ala   | Gly   | Arg | Phe   | Asp   | Ile   | Trp   | Ala   | Pro | Glu |       |  |
| 1   |       |     |       | 5   |       |       |       |     | 1 0   |       |       |       |       | 1 5 |     |       |  |
| GCA | GGC   | ACC | GTA   | ACG | CTG   | CTG   | GCC   | GAC | GGG   | GAG   | CAC   | TAC   | GAG   | ATG | GGC | 9 6   |  |
| Ala | Gly   | Thr | Val   | Thr | Leu   | Leu   | Ala   | Gly | Gly   | Glu   | Arg   | Tyr   | Glu   | Met | Gly |       |  |
|     |       |     | 2 0   |     |       |       | 2 5   |     |       |       |       | 3 0   |       |     |     |       |  |
| CGC | CGC   | CCC | GGC   | AAC | GGG   | CCG   | GCG   | GAC | GAA   | GGC   | TGG   | TGG   | ACG   | GCC | GCG | 1 4 4 |  |
| Arg | Arg   | Pro | Gly   | Asn | Gly   | Pro   | Ala   | Asp | Glu   | Gly   | Trp   | Trp   | Thr   | Ala | Ala |       |  |
|     |       |     | 3 5   |     |       | 4 0   |       | 4 0 |       |       | 4 5   |       |       |     |     |       |  |
| GAT | GCA   | CCG | ACA   | GGC | GCG   | GAC   | GTG   | GAC | TAC   | GGA   | TAC   | CTG   | CTC   | GAC | GGC | 1 9 2 |  |
| Asp | Ala   | Pro | Thr   | Gly | Ala   | Asp   | 5 0   | Val | Asp   | Tyr   | Gly   | Tyr   | Leu   | Leu | Asp | Gly   |  |
|     |       |     |       |     | 5 5   |       |       |     |       | 6 0   |       |       |       |     |     |       |  |
| GAC | GAA   | ATC | CCG   | CTG | CCG   | GAC   | CCC   | CGG | ACC   | CGC   | CGC   | CAG   | CCC   | GAA | GGC | 2 4 0 |  |
| Asp | Glu   | Ile | Pro   | Leu | Pro   | Asp   | 7 0   | Pro | Arg   | Thr   | Arg   | Gln   | Pro   | Glu | Gly |       |  |
|     |       |     |       |     |       |       |       | 7 0 |       | 7 5   |       |       |       |     |     | 8 0   |  |
| GTC | CAT   | GCC | CTG   | TCC | CGG   | ACC   | TTC   | GAC | CCC   | GGC   | GCC   | CAC   | CGC   | TGG | CAG | 2 8 8 |  |
| Val | His   | Ala | Leu   | Ser | Arg   | Thr   | Phe   | Asp | Pro   | Gly   | Ala   | His   | Arg   | Trp | Gln |       |  |
|     |       |     |       | 8 5 |       |       |       | 9 0 |       |       |       |       | 9 5   |     |     |       |  |
| GAC | GCC   | GGG | TGG   | CAG | GGC   | AGG   | GAA   | CAC | CAG   | GGC   | TCC   | GTC   | ATT   | TAC | GAA | 3 3 6 |  |
| Asp | Ala   | Gly | Trp   | Gln | Gly   | Arg   | Glu   | Leu | Gln   | Gly   | Ser   | Val   | Ile   | Tyr | Glu |       |  |
|     |       |     | 1 0 0 |     |       |       | 1 0 5 |     |       |       |       | 1 1 0 |       |     |     |       |  |
| CTC | CAC   | ATC | GGA   | ACG | TTC   | ACG   | CCG   | GAA | GGG   | ACG   | CTG   | GAC   | GCC   | GCC | GCG | 3 8 4 |  |
| Leu | His   | Ile | Gly   | Thr | Phe   | Thr   | Pro   | Glu | Gly   | Thr   | Leu   | Asp   | Ala   | Ala | Ala |       |  |
|     |       |     | 1 1 5 |     |       | 1 2 0 |       |     |       |       | 1 2 5 |       |       |     |     |       |  |
| GGC | AAG   | CTG | GAC   | TAC | CTC   | GCC   | GGC   | CTG | GGC   | ATC   | GAC   | TTC   | ATT   | GAG | CTG | 4 3 2 |  |
| Gly | Lys   | Leu | Asp   | Tyr | Leu   | Ala   | 1 3 0 | Gly | Leu   | Gly   | Ile   | Asp   | Phe   | Ile | Glu |       |  |
|     |       |     |       |     |       | 1 3 5 |       |     |       | 1 4 0 |       |       |       |     |     |       |  |
| CTG | CCC   | GTG | AAT   | GCC | TTC   | AAC   | GGC   | ACG | CAC   | AAAC  | TGG   | GGC   | TAC   | GAC | GGC | 4 8 0 |  |
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|     |       |     | 1 4 5 |     |       | 1 5 0 |       |     |       | 1 5 5 |       |       |       |     |     | 1 6 0 |  |
| GTC | CAG   | TGG | TTT   | GCC | GTG   | CAT   | GAA   | GGC | TAC   | GGC   | GGG   | CCT   | GCG   | GCG | TAC | 5 2 8 |  |
| Val | Gln   | Trp | Phe   | Ala | Val   | His   | Glu   | Gly | Tyr   | Gly   | Gly   | Pro   | Ala   | Ala | Tyr |       |  |
|     |       |     | 1 6 5 |     |       |       |       |     | 1 7 0 |       |       |       | 1 7 5 |     |     |       |  |
| CAG | CGG   | TTC | GTG   | GAT | GCG   | GCC   | CAC   | GCG | GCC   | GCG   | CTC   | GGC   | GTC   | ATC | CAG | 5 7 6 |  |
| Gln | Arg   | Phe | Val   | Asp | Ala   | Ala   | His   | Ala | Ala   | Gly   | Leu   | Gly   | Val   | Ile | Gln |       |  |
|     |       |     | 1 8 0 |     |       |       | 1 8 5 |     |       |       |       |       | 1 9 0 |     |     |       |  |
| GAC | GTG   | GTC | TAC   | AAC | CAC   | CTC   | GGG   | CCG | AGC   | GGG   | AAC   | TAC   | CTC   | CCC | AGG | 6 2 4 |  |
| Asp | Val   | Val | Tyr   | Asn | His   | Leu   | Gly   | Pro | Ser   | Gly   | Asn   | Tyr   | Leu   | Pro | Arg |       |  |
|     |       |     | 1 9 5 |     |       |       | 2 0 0 |     |       |       |       | 2 0 5 |       |     |     |       |  |
| TAC | GGC   | CCG | TAC   | CTC | AAG   | CAC   | GGC   | GAA | GGC   | AAC   | ACC   | TGG   | GGC   | GAT | TCG | 6 7 2 |  |
| Tyr | Gly   | Pro | Tyr   | Leu | Lys   | His   | Gly   | Glu | Gly   | Asn   | Thr   | Trp   | Gly   | Asp | Ser |       |  |
|     | 2 1 0 |     |       |     | 2 1 5 |       |       |     |       |       | 2 2 0 |       |       |     |     |       |  |

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|       |     |     |     |       |     |       |       |       |       |       |       |       |       |       |       |     |         |         |
|-------|-----|-----|-----|-------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|---------|---------|
| GTC   | AAC | CTG | GAC | GGG   | CCG | GGA   | TCC   | GAC   | CAC   | GTC   | CGC   | CAG   | TAC   | ATC   | CTG   |     | 7 2 0   |         |
| Val   | Asn | Leu | Asp | Gly   | Pro | Gly   | Ser   | Asp   | His   | Val   | Arg   | Gln   | Tyr   | Ile   | Leu   |     |         |         |
| 2 2 5 |     |     |     | 2 3 0 |     |       |       |       | 2 3 5 |       |       |       |       |       | 2 4 0 |     |         |         |
| GAC   | AAC | GTG | GCC | ATG   | TGG | CTG   | CGC   | GAC   | TAC   | CGG   | GTG   | GAC   | GGC   | CTC   | CGC   |     | 7 6 8   |         |
| Asp   | Asn | Val | Ala | Met   | Trp | Leu   | Arg   | Asp   | Tyr   | Arg   | Val   | Asp   | Gly   | Leu   | Arg   |     |         |         |
|       |     |     |     | 2 4 5 |     |       |       |       | 2 5 0 |       |       |       |       | 2 5 5 |       |     |         |         |
| CTG   | GAC | GCC | GTC | CAC   | GCC | CTG   | AAG   | GAT   | GAG   | CGG   | GCC   | GTC   | CAC   | ATC   | CTG   |     | 8 1 6   |         |
| Leu   | Asp | Ala | Val | His   | Ala | Leu   | Lys   | Asp   | 2 6 5 | Arg   | Ala   | Val   | His   | Ile   | Leu   |     |         |         |
|       |     |     |     | 2 6 0 |     |       |       |       |       |       | 2 7 0 |       |       |       |       |     |         |         |
| GAG   | GAG | TTC | GGC | GCG   | CTG | GCG   | GAC   | GCC   | CTG   | TCG   | TCC   | GAA   | GGC   | GGC   | CGC   |     | 8 6 4   |         |
| Glu   | Glu | Phe | Gly | Ala   | Leu | Ala   | Asp   | Ala   | Leu   | Ser   | Ser   | Glu   | Gly   | Gly   | Arg   |     |         |         |
|       |     |     |     | 2 7 5 |     |       |       | 2 8 0 |       |       | 2 8 5 |       |       |       |       |     |         |         |
| CCG   | CTG | ACC | CTC | ATC   | GCC | GAG   | TCC   | GAC   | CTC   | AAC   | AAT   | CCG   | CGG   | CTG   | CTG   |     | 9 1 2   |         |
| Pro   | Leu | Thr | Leu | Ile   | Ala | Glu   | Ser   | Asp   | Leu   | Asn   | Asn   | Pro   | Arg   | Leu   | Leu   |     |         |         |
|       |     |     |     | 2 9 0 |     | 2 9 5 |       |       | 3 0 0 |       |       |       |       |       |       |     |         |         |
| TAC   | CCC | CGG | GAT | GTC   | AAC | GGC   | TAC   | GGA   | CTG   | GCC   | GGC   | CAG   | TGG   | AGC   | GAC   |     | 9 6 0   |         |
| Tyr   | Pro | Arg | Asp | Val   | Asn | Gly   | Tyr   | Gly   | Leu   | Ala   | Gly   | Gln   | Trp   | Ser   | Asp   |     |         |         |
|       |     |     |     | 3 1 0 |     |       |       |       | 3 1 5 |       |       |       | 3 2 0 |       |       |     |         |         |
| GAC   | TTC | CAC | CAC | GCC   | GTG | CAC   | GTC   | AAC   | GTC   | AGC   | GGG   | GAA   | ACC   | ACC   | GGC   |     | 1 0 0 8 |         |
| Asp   | Phe | His | His | Ala   | Val | His   | Val   | Asn   | Val   | Ser   | Gly   | Glu   | Thr   | Thr   | Gly   |     |         |         |
|       |     |     |     | 3 2 5 |     |       |       | 3 3 0 |       |       |       | 3 3 5 |       |       |       |     |         |         |
| TAC   | TAC | AGC | GAC | TTC   | GAC | TCG   | CTC   | GGA   | GCC   | CTC   | GCC   | AAG   | GTC   | CTG   | CGT   |     | 1 0 5 6 |         |
| Tyr   | Tyr | Ser | Asp | Phe   | Asp | Ser   | Leu   | Gly   | Ala   | Leu   | Ala   | Lys   | Val   | Leu   | Arg   |     |         |         |
|       |     |     |     | 3 4 0 |     |       |       | 3 4 5 |       |       |       | 3 5 0 |       |       |       |     |         |         |
| GAC   | GGG | TTC | TTC | CAC   | GAC | GGC   | AGC   | TAC   | TCC   | AGC   | TTC   | CGC   | GGC   | CGC   | TGC   |     | 1 1 0 4 |         |
| Asp   | Gly | Phe | Phe | His   | Asp | Gly   | Ser   | Tyr   | Ser   | Ser   | Phe   | Arg   | Gly   | Arg   | Cys   |     |         |         |
|       |     |     |     | 3 5 5 |     |       | 3 6 0 |       |       |       | 3 6 5 |       |       |       |       |     |         |         |
| CAC   | GGC | CGG | CCG | ATC   | AAC | TTC   | AGC   | GCC   | GTG   | CAT   | CCG   | GCC   | GCG   | CTG   | GTG   |     | 1 1 5 2 |         |
| His   | Gly | Arg | Pro | Ile   | Asn | Phe   | Ser   | Ala   | Val   | His   | Pro   | Ala   | Ala   | Leu   | Val   |     |         |         |
|       |     |     |     | 3 7 0 |     | 3 7 5 |       |       |       | 3 8 0 |       |       |       |       |       |     |         |         |
| GTC   | TGC | TCA | CAG | AAC   | CAT | GAC   | CAG   | ATC   | GGC   | AAC   | CGG   | GCC   | ACC   | GGG   | GAC   |     | 1 2 0 0 |         |
| Val   | Cys | Ser | Gln | Asn   | His | Asp   | Gln   | Ile   | Gly   | Asn   | Arg   | Ala   | Thr   | Gly   | Asp   |     |         |         |
|       |     |     |     | 3 8 5 |     | 3 9 0 |       |       |       | 3 9 5 |       |       |       | 4 0 0 |       |     |         |         |
| CGG   | CTG | TCC | CAG | TCA   | CTT | CCG   | TAC   | GGA   | AGC   | CTG   | GCC   | CTG   | GCC   | GCC   | GTG   |     | 1 2 4 8 |         |
| Arg   | Leu | Ser | Gln | Ser   | Leu | Pro   | Tyr   | Gly   | Ser   | Leu   | Ala   | Leu   | Ala   | Ala   | Val   |     |         |         |
|       |     |     |     | 4 0 5 |     |       |       | 4 1 0 |       |       |       |       |       | 4 1 5 |       |     |         |         |
| CTG   | ACC | CTC | ACC | GGT   | CCG | TTC   | ACG   | CCC   | ATG   | CTG   | TTC   | ATG   | GGA   | GAG   | GAA   |     | 1 2 9 6 |         |
| Leu   | Thr | Leu | Thr | Gly   | Pro | Phe   | Thr   | Pro   | Met   | Leu   | Phe   | Met   | Gly   | Glu   | Glu   |     |         |         |
|       |     |     |     | 4 2 0 |     |       |       | 4 2 5 |       |       |       |       | 4 3 0 |       |       |     |         |         |
| TAC   | GGG | GCC | ACC | ACC   | CCG | TGG   | CAG   | TTC   | TTC   | ACC   | TCG   | CAC   | CCT   | GAA   | CCC   |     | 1 3 4 4 |         |
| Tyr   | Gly | Ala | Thr | Thr   | Pro | Trp   | Gln   | Phe   | Phe   | Thr   | Ser   | His   | Pro   | Glu   | Pro   |     |         |         |
|       |     |     |     | 4 3 5 |     |       | 4 4 0 |       |       |       | 4 4 5 |       |       |       |       |     |         |         |
| GAG   | CTG | GGC | AAG | GCC   | ACG | GCC   | GAG   | GGA   | GGC   | AGG   | ATC   | AGG   | GAG   | TTC   | GAG   | CGC |         | 1 3 9 2 |
| Glu   | Leu | Gly | Lys | Ala   | Thr | Ala   | Glu   | Gly   | Arg   | Ile   | Arg   | 4 6 0 |       |       |       |     |         |         |
|       |     |     |     | 4 5 0 |     |       | 4 5 5 |       |       |       |       |       |       |       |       |     |         |         |
| ATG   | GGG | TGG | GAT | CCC   | GCC | GTC   | GTG   | CCC   | GAT   | CCG   | CAG   | GAT   | CCG   | GAG   | ACC   |     | 1 4 4 0 |         |
| Met   | Gly | Trp | Asp | Pro   | Ala | Val   | Val   | Pro   | Asp   | Pro   | Gln   | Asp   | Pro   | Glu   | Thr   |     |         |         |
|       |     |     |     | 4 6 5 |     | 4 7 0 |       |       |       | 4 7 5 |       |       |       | 4 8 0 |       |     |         |         |
| TTC   | ACC | CGC | TCC | AAA   | CTG | GAC   | TGG   | GCG   | GAA   | GCG   | TCC   | GCC   | GGC   | GAT   | CAT   |     | 1 4 8 8 |         |
| Phe   | Thr | Arg | Ser | Lys   | Leu | Asp   | Trp   | Ala   | Glu   | Ala   | Ser   | Ala   | Gly   | Asp   | His   |     |         |         |
|       |     |     |     | 4 8 5 |     |       |       | 4 9 0 |       |       |       |       |       | 4 9 5 |       |     |         |         |
| GCC   | CGC | CTC | CTG | GAG   | CTG | TAC   | CGC   | TCG   | CTT   | ATC   | ACG   | CTG   | CGG   | CGG   | TCA   |     | 1 5 3 6 |         |
| Ala   | Arg | Leu | Leu | Glu   | Leu | Tyr   | Arg   | Ser   | Leu   | Ile   | Thr   | Leu   | Arg   | Arg   | Ser   |     |         |         |
|       |     |     |     | 5 0 0 |     |       | 5 0 5 |       |       |       |       |       | 5 1 0 |       |       |     |         |         |
| ACT   | CCG | GAG | CTC | GCG   | CGC | CTG   | GGC   | TTT   | GCG   | GAC   | ACC   | GCC   | GTC   | GAG   | TTC   |     | 1 5 8 4 |         |
| Thr   | Pro | Glu | Leu | Ala   | Arg | Leu   | Gly   | Phe   | Ala   | Asp   | Thr   | Ala   | Val   | Glu   | Phe   |     |         |         |
|       |     |     |     | 5 1 5 |     |       | 5 2 0 |       |       |       | 5 2 5 |       |       |       |       |     |         |         |
| GAC   | GAC | GAC | GCC | CGC   | TGG | CTC   | CGT   | TAT   | TGG   | CGC   | GGA   | GGC   | GTG   | CAG   | GTG   |     | 1 6 3 2 |         |
| Asp   | Asp | Asp | Ala | Arg   | Trp | Leu   | Arg   | Tyr   | Trp   | Arg   | 5 3 0 |       | 5 4 0 |       | Val   |     |         |         |

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| GTG | CTG | AAC | TTC | GCG | GAC | CGT | CCC | ATC | AGC | CTG | GAC | CGG | CCG | GGA | ACC |     | 1680 |
| Val | Leu | Asn | Phe | Ala | Asp | Arg | Pro | Ile | Ser | Leu | Asp | Arg | Pro | Gly | Thr |     |      |
| 545 |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     |     |     | 560 |      |
| GCG | CTG | CTG | CTC | GCC | ACC | GAC | GAC | GCC | GTC | CGG | ATG | GAC | GGA | GTC | CAG |     | 1728 |
| Ala | Leu | Leu | Leu | Ala | Thr | Asp | Asp | Ala | Val | Arg | Met | Asp | Gly | Val | Gln |     |      |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     |     |     | 575 |      |
| GTG | GAG | CTG | CCG | CCG | CTG | AGC | GCC | GCG | GTT | CTG | CGC | GAC |     |     |     |     | 1767 |
| Val | Glu | Leu | Pro | Pro | Leu | Ser | Ala | Ala | Val | Leu | Arg | Asp |     |     |     |     |      |
|     |     |     |     | 580 |     |     |     |     | 585 |     |     |     |     |     |     |     |      |

## ( 2 ) INFORMATION FOR SEQ ID NO:2:

## ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 589 amino acids
- ( B ) TYPE: amino acid
- ( D ) TOPOLOGY: linear

## ( ii ) MOLECULE TYPE: protein

## ( xi ) SEQUENCE DESCRIPTION: SEQ ID NO:2:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ala | Lys | Pro | Val | Gln | Gly | Ala | Gly | Arg | Phe | Asp | Ile | Trp | Ala | Pro | Glu |     |  |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     |     |     | 15  |  |
| Ala | Gly | Thr | Val | Thr | Leu | Leu | Ala | Gly | Gly | Glu | Arg | Tyr | Glu | Met | Gly |     |  |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     |     |     | 30  |     |  |
| Arg | Arg | Pro | Gly | Asn | Gly | Pro | Ala | Asp | Glu | Gly | Trp | Trp | Thr | Ala | Ala |     |  |
| 35  |     |     |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |     |  |
| Asp | Ala | Pro | Thr | Gly | Ala | Asp | Val | Asp | Tyr | Gly | Tyr | Leu | Leu | Asp | Gly |     |  |
| 50  |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |     |     |  |
| Asp | Glu | Ile | Pro | Leu | Pro | Asp | Pro | Arg | Thr | Arg | Gln | Pro | Glu | Gly |     |     |  |
| 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     |     |     | 80  |  |
| Val | His | Ala | Leu | Ser | Arg | Thr | Phe | Asp | Pro | Gly | Ala | His | Arg | Trp | Gln |     |  |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     |     |     |  |
| Asp | Ala | Gly | Trp | Gln | Gly | Arg | Glu | Leu | Gln | Gly | Ser | Val | Ile | Tyr | Glu |     |  |
|     |     |     | 100 |     |     |     | 105 |     |     |     |     |     |     |     |     | 110 |  |
| Leu | His | Ile | Gly | Thr | Phe | Thr | Pro | Glu | Gly | Thr | Leu | Asp | Ala | Ala | Ala |     |  |
| 115 |     |     |     |     | 120 |     |     |     |     |     |     | 125 |     |     |     |     |  |
| Gly | Lys | Leu | Asp | Tyr | Leu | Ala | Gly | Leu | Gly | Ile | Asp | Phe | Ile | Glu | Leu |     |  |
| 130 |     |     |     | 135 |     |     |     |     |     | 140 |     |     |     |     |     |     |  |
| Leu | Pro | Val | Asn | Ala | Phe | Asn | Gly | Thr | His | Asn | Trp | Gly | Tyr | Asp | Gly |     |  |
| 145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     |     |     | 160 |  |
| Val | Gln | Trp | Phe | Ala | Val | His | Glu | Gly | Tyr | Gly | Gly | Pro | Ala | Ala | Tyr |     |  |
|     |     |     | 165 |     |     |     |     | 170 |     |     |     |     |     |     |     | 175 |  |
| Gln | Arg | Phe | Val | Asp | Ala | Ala | His | Ala | Ala | Gly | Leu | Gly | Val | Ile | Gln |     |  |
|     |     |     | 180 |     |     |     | 185 |     |     |     |     |     |     |     |     |     |  |
| Asp | Val | Val | Tyr | Asn | His | Leu | Gly | Pro | Ser | Gly | Asn | Tyr | Leu | Pro | Arg |     |  |
| 195 |     |     |     |     | 200 |     |     |     |     |     |     | 205 |     |     |     |     |  |
| Tyr | Gly | Pro | Tyr | Leu | Lys | His | Gly | Glu | Gly | Asn | Thr | Trp | Gly | Asp | Ser |     |  |
| 210 |     |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |     |     |  |
| Val | Asn | Leu | Asp | Gly | Pro | Gly | Ser | Asp | His | Val | Arg | Gln | Tyr | Ile | Leu |     |  |
| 225 |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     |     |     | 240 |  |
| Asp | Asn | Val | Ala | Met | Trp | Leu | Arg | Asp | Tyr | Arg | Val | Asp | Gly | Leu | Arg |     |  |
|     |     |     | 245 |     |     |     | 250 |     |     |     |     |     |     |     |     | 255 |  |
| Leu | Asp | Ala | Val | His | Ala | Leu | Lys | Asp | Glu | Arg | Ala | Val | His | Ile | Leu |     |  |
| 260 |     |     |     |     |     | 265 |     |     |     |     |     |     |     |     |     |     |  |
| Glu | Glu | Phe | Gly | Ala | Leu | Ala | Asp | Ala | Leu | Ser | Ser | Glu | Gly | Gly | Arg |     |  |
|     |     |     | 275 |     |     |     | 280 |     |     |     |     | 285 |     |     |     |     |  |
| Pro | Leu | Thr | Leu | Ile | Ala | Glu | Ser | Asp | Leu | Asn | Asn | Pro | Arg | Leu | Leu |     |  |
| 290 |     |     |     | 295 |     |     |     |     |     | 300 |     |     |     |     |     |     |  |

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|      |      |      |     |      |     |     |      |     |      |     |     |      |     |      |      |
|------|------|------|-----|------|-----|-----|------|-----|------|-----|-----|------|-----|------|------|
| Tyr  | Pro  | Arg  | Asp | Val  | Asn | Gly | Tyr  | Gly | Leu  | Ala | Gly | Gln  | Trp | Ser  | Asp  |
| 305  |      |      |     | 310  |     |     |      |     | 315  |     |     |      |     |      | 320  |
| Asp  | Phe  | His  | His | Ala  | Val | His | Val  | Asn | Val  | Ser | Gly | Glu  | Thr | Thr  | Gly  |
|      | 325  |      |     |      |     |     |      | 330 |      |     |     |      | 335 |      |      |
| Tyr  | Tyr  | Ser  | Asp | Phe  | Asp | Ser | Leu  | Gly | Ala  | Leu | Ala | Lys  | Val | Leu  | Arg  |
|      |      |      | 340 |      |     |     |      | 345 |      |     |     |      | 350 |      |      |
| Asp  | Gly  | Phe  | Phe | His  | Asp | Gly | Ser  | Tyr | Ser  | Ser | Phe | Arg  | Gly | Arg  | Cys  |
|      | 355  |      |     |      |     | 360 |      |     |      |     | 365 |      |     |      |      |
| His  | Gly  | Arg  | Pro | Ile  | Asn | Phe | Ser  | Ala | Val  | His | Pro | Ala  | Ala | Leu  | Val  |
|      | 370  |      |     |      | 375 |     |      |     |      | 380 |     |      |     |      |      |
| Val  | Cys  | Ser  | Gln | Asn  | His | Asp | Gln  | Ile | Gly  | Asn | Arg | Ala  | Thr | Gly  | Asp  |
|      | 385  |      |     |      | 390 |     |      |     | 395  |     |     |      |     | 400  |      |
| Arg  | Leu  | Ser  | Gln | Ser  | Leu | Pro | Tyr  | Gly | Ser  | Leu | Ala | Leu  | Ala | Ala  | Val  |
|      | 405  |      |     |      |     |     | 410  |     |      |     |     |      | 415 |      |      |
| Leu  | Thr  | Leu  | Thr | Gly  | Pro | Phe | Thr  | Pro | Met  | Leu | Phe | Met  | Gly | Glut | Glut |
|      | 420  |      |     |      |     | 425 |      |     |      |     |     |      | 430 |      |      |
| Tyr  | Gly  | Ala  | Thr | Thr  | Pro | Trp | Gln  | Phe | Phe  | Thr | Ser | His  | Pro | Glut | Pro  |
|      | 435  |      |     |      |     | 440 |      |     |      |     | 445 |      |     |      |      |
| Glut | Leu  | Gly  | Lys | Ala  | Thr | Ala | Glut | Gly | Arg  | Ile | Arg | Glut | Phe | Glut | Arg  |
|      | 450  |      |     |      | 455 |     |      |     | 460  |     |     |      |     |      |      |
| Met  | Gly  | Trp  | Asp | Pro  | Ala | Val | Val  | Pro | Asp  | Pro | Gln | Asp  | Pro | Glut | Thr  |
|      | 465  |      |     | 470  |     |     |      |     | 475  |     |     |      |     |      | 480  |
| Phe  | Thr  | Arg  | Ser | Lys  | Leu | Asp | Trp  | Ala | Glut | Ala | Ser | Ala  | Gly | Asp  | His  |
|      | 485  |      |     |      |     | 490 |      |     |      |     |     |      | 495 |      |      |
| Ala  | Arg  | Leu  | Leu | Glut | Leu | Tyr | Arg  | Ser | Leu  | Ile | Thr | Leu  | Arg | Arg  | Ser  |
|      | 500  |      |     |      |     | 505 |      |     |      |     |     |      | 510 |      |      |
| Thr  | Pro  | Glut | Leu | Ala  | Arg | Leu | Gly  | Phe | Ala  | Asp | Thr | Ala  | Val | Glut | Phe  |
|      | 515  |      |     |      |     | 520 |      |     |      |     |     |      |     |      |      |
| Asp  | Asp  | Asp  | Ala | Arg  | Trp | Leu | Arg  | Tyr | Trp  | Arg | Gly | Gly  | Val | Gln  | Val  |
|      | 530  |      |     |      |     | 535 |      |     |      |     | 540 |      |     |      |      |
| Val  | Leu  | Asn  | Phe | Ala  | Asp | Arg | Pro  | Ile | Ser  | Leu | Asp | Arg  | Pro | Gly  | Thr  |
|      | 545  |      |     | 550  |     |     |      |     | 555  |     |     |      |     |      | 560  |
| Ala  | Leu  | Leu  | Leu | Ala  | Thr | Asp | Asp  | Ala | Val  | Arg | Met | Asp  | Gly | Val  | Gln  |
|      | 565  |      |     |      |     |     |      | 570 |      |     |     |      |     | 575  |      |
| Val  | Glut | Leu  | Pro | Pro  | Leu | Ser | Ala  | Ala | Val  | Leu | Arg | Asp  |     |      |      |
|      | 580  |      |     |      |     |     | 585  |     |      |     |     |      |     |      |      |

( 2 ) INFORMATION FOR SEQ ID NO:3:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 1791 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( i x ) FEATURE:

- ( A ) NAME/KEY: CDS
- ( B ) LOCATION: 1..1791

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:3:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| ACG | CAC | ACC | TAC | CCG | CGG | GAA | GCC | GCG | AAA | CCC | GTC | CTG | GGC | CCC | GCA | 48 |
| Thr | His | Thr | Tyr | Pro | Arg | Glu | Ala | Ala | Lys | Pro | Val | Leu | Gly | Pro | Ala |    |
| 590 |     |     |     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |    |
| CGC | TAC | GAC | GTC | TGG | GCG | CCC | AAC | GCT | GAA | TCC | GTG | ACG | CTG | CTG | GCC | 96 |
| Arg | Tyr | Asp | Val | Trp | Ala | Pro | Asn | Ala | Glu | Ser | Val | Thr | Leu | Leu | Ala |    |
|     |     |     |     |     |     |     |     |     | 615 |     |     |     |     |     | 620 |    |

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|       |     |     |      |       |     |       |       |     |     |       |       |       |       |       |     |     |         |       |
|-------|-----|-----|------|-------|-----|-------|-------|-----|-----|-------|-------|-------|-------|-------|-----|-----|---------|-------|
| GGC   | GGG | GAG | CGC  | TAC   | GCC | ATG   | CAG   | CGC | CGG | GCC   | GAG   | ACC   | GGG   | CCG   | GAG |     | 1 4 4   |       |
| Gly   | Gly | Glu | Arg  | Tyr   | Ala | Met   | Gln   | Arg | Arg | Ala   | Glu   | Thr   | Gly   | Pro   | Glu |     |         |       |
| 6 2 5 |     |     |      |       |     |       | 6 3 0 |     |     |       |       |       | 6 3 5 |       |     |     |         |       |
| GAC   | GCC | GGC | TGG  | TGG   | ACC | GCC   | GCC   | GCG | GCG | CCT   | ACG   | GAT   | GGC   | AAC   | GTG |     | 1 9 2   |       |
| Asp   | Ala | Gly | Trp  | Trp   | Thr | Ala   | Ala   | Gly | Ala | Pro   | Thr   | Asp   | Gly   | Asn   | Val |     |         |       |
| 6 4 0 |     |     |      |       |     |       | 6 4 5 |     |     |       |       | 6 5 0 |       |       |     |     |         |       |
| GAC   | TAC | GGG | TAC  | CTT   | CTG | GAC   | GGC   | GAC | GAA | ACA   | CCG   | CTT   | CCG   | GAT   | CCA |     | 2 4 0   |       |
| Asp   | Tyr | Gly | Tyr  | Leu   | Leu | Asp   | Gly   | Asp | Glu | Thr   | Pro   | Pro   | Leu   | Pro   | Asp | Pro |         |       |
| 6 5 5 |     |     |      |       |     | 6 6 0 |       |     |     |       | 6 6 5 |       |       |       |     |     |         |       |
| CGG   | ACC | CGC | CGC  | CAG   | CCC | GAC   | GGC   | GTC | CAC | GCC   | CTG   | TCC   | CGC   | ACG   | TTT |     | 2 8 8   |       |
| Arg   | Thr | Arg | Arg  | Gln   | Pro | Asp   | Gly   | Val | His | Ala   | Leu   | Ser   | Arg   | Thr   | Phe |     |         |       |
| 6 7 0 |     |     |      | 6 7 5 |     |       |       |     |     | 6 8 0 |       |       |       |       |     |     |         |       |
| GAC   | CCG | TCC | GCG  | TAC   | AGC | TGG   | CAG   | GAC | GAC | GCC   | TGG   | CAG   | GGC   | AGG   | GAA |     | 3 3 6   |       |
| Asp   | Pro | Ser | Ala  | Tyr   | Ser | Trp   | Gln   | Asp | Asp | Ala   | Trp   | Gln   | Gly   | Arg   | Glu |     |         |       |
| 6 9 0 |     |     |      |       |     | 6 9 5 |       |     |     |       | 7 0 0 |       |       |       |     |     |         |       |
| CTG   | CAG | GGC | GCC  | GTC   | ATC | TAC   | GAG   | CTC | CAC | CTC   | GGA   | ACA   | TTC   | ACG   | CCC |     | 3 8 4   |       |
| Leu   | Gln | Gly | Ala  | Val   | Ile | Tyr   | Glu   | Leu | His | Leu   | Gly   | Thr   | Phe   | Thr   | Pro |     |         |       |
| 7 0 5 |     |     |      |       |     |       | 7 1 0 |     |     |       |       | 7 1 5 |       |       |     |     |         |       |
| GAA   | GGG | ACG | CTG  | GAG   | GCG | GCC   | GCC   | GGA | AAG | CTG   | GAC   | TAC   | CTC   | GCC   | GGC |     | 4 3 2   |       |
| Glu   | Gly | Thr | Leu  | Glu   | Ala | Ala   | Ala   | Gly | Lys | Leu   | Asp   | Tyr   | Leu   | Ala   | Gly |     |         |       |
| 7 2 0 |     |     |      |       |     | 7 2 5 |       |     |     |       | 7 3 0 |       |       |       |     |     |         |       |
| TTG   | GGC | GTC | GAC  | TTC   | ATC | GAG   | CTG   | CTG | CCG | GTG   | AAC   | GCT   | TTC   | AAc   | GGC |     | 4 8 0   |       |
| Leu   | Gly | Val | Asp  | Phe   | Ile | Glu   | Leu   | Leu | Pro | Val   | Asn   | Ala   | Phe   | Asn   | Gly |     |         |       |
| 7 3 5 |     |     |      | 7 4 0 |     |       |       |     |     | 7 4 5 |       |       |       |       |     |     |         |       |
| ACG   | CAC | AAC | TGG  | GGT   | TAC | GAC   | GGT   | Gly | GTC | CAG   | TGG   | TTC   | GCT   | GTG   | CAC | GAG |         | 5 2 8 |
| Thr   | His | Asn | Trp  | Gly   | Tyr | Asp   | Gly   | Asp | Val | Gln   | Trp   | Phe   | Ala   | Val   | His | Glu |         |       |
| 7 5 0 |     |     |      | 7 5 5 |     |       |       |     |     | 7 6 0 |       |       |       |       |     |     |         |       |
| GCA   | TAC | GGC | GGG  | CCG   | GAA | GCG   | TAC   | CAG | CGG | TTC   | GTC   | GAC   | GCC   | GCC   | CAC |     | 5 7 6   |       |
| Ala   | Tyr | Gly | Gly  | Pro   | Glu | Ala   | Tyr   | Gln | Arg | Phe   | Val   | Asp   | Ala   | Ala   | His |     |         |       |
| 7 7 0 |     |     |      |       |     | 7 7 5 |       |     |     |       | 7 8 0 |       |       |       |     |     |         |       |
| GCC   | GCA | GGC | CTT  | GGC   | GTC | ATC   | CAG   | GAC | GTG | GTC   | TAC   | AAC   | CAC   | CTC   | GGC |     | 6 2 4   |       |
| Ala   | Ala | Gly | Leu  | Gly   | Val | Ile   | Gln   | Asp | Val | Val   | Tyr   | Asn   | His   | Leu   | Gly |     |         |       |
| 7 8 5 |     |     |      |       |     | 7 9 0 |       |     |     |       | 7 9 5 |       |       |       |     |     |         |       |
| CCC   | AGC | GGG | AAC  | TAC   | CTG | CCG   | CGG   | TTC | GGG | CCG   | TAC   | CTC   | AAG   | CAG   | GGC |     | 6 7 2   |       |
| Pro   | Ser | Gly | Asn  | Tyr   | Leu | Pro   | Arg   | Phe | Gly | Pro   | Tyr   | Leu   | Lys   | Gln   | Gly |     |         |       |
| 8 0 0 |     |     |      |       |     | 8 0 5 |       |     |     |       | 8 1 0 |       |       |       |     |     |         |       |
| GAG   | GGT | AAC | ACG  | TGG   | GGC | GAC   | TCG   | GTG | AAC | CTG   | GAC   | GGG   | CCC   | GGC   | TCC |     | 7 2 0   |       |
| Glu   | Gly | Asn | Thr  | Trp   | Gly | Asp   | Ser   | Val | Asn | Leu   | Asp   | Gly   | Pro   | Gly   | Ser |     |         |       |
| 8 1 5 |     |     |      |       |     | 8 2 0 |       |     |     |       | 8 2 5 |       |       |       |     |     |         |       |
| GAC   | CAT | GTG | CGC  | CGG   | TAC | ATC   | CTG   | GAC | AAC | CTG   | GCC   | ATG   | TGG   | CTG   | CGT |     | 7 6 8   |       |
| Asp   | His | Val | Arg  | Arg   | Tyr | Ile   | Leu   | Asp | Asn | Leu   | Ala   | Met   | Trp   | Leu   | Arg |     |         |       |
| 8 3 0 |     |     |      | 8 3 5 |     |       |       |     |     | 8 4 0 |       |       |       |       |     |     |         |       |
| GAC   | TAC | CGG | G TG | GAC   | GGC | CTG   | CGG   | CTG | GAC | GCC   | GTC   | CAC   | GCC   | CTG   | AAG |     | 8 1 6   |       |
| Asp   | Tyr | Arg | Val  | Asp   | Gly | Leu   | Arg   | Leu | Asp | Ala   | Val   | His   | Ala   | Leu   | Lys |     |         |       |
| 8 5 0 |     |     |      |       |     | 8 5 5 |       |     |     |       | 8 6 0 |       |       |       |     |     |         |       |
| GAT   | GAG | CGG | GCG  | GTG   | CAC | ATC   | CTG   | GAG | GAC | TTC   | GGG   | GCG   | CTG   | GCC   | GAT |     | 8 6 4   |       |
| Asp   | Glu | Arg | Ala  | Val   | His | Ile   | Leu   | Glu | Asp | Phe   | Gly   | Ala   | Leu   | Ala   | Asp |     |         |       |
| 8 6 5 |     |     |      |       |     | 8 7 0 |       |     |     |       | 8 7 5 |       |       |       |     |     |         |       |
| CAG   | ATC | TCC | GCC  | GAG   | GTG | GGA   | CGG   | CCG | CTG | ACG   | CTC   | ATC   | GCC   | GAG   | TCC |     | 9 1 2   |       |
| Gln   | Ile | Ser | Ala  | Glu   | Val | Gly   | Arg   | Pro | Leu | Thr   | Leu   | Ile   | Ala   | Glu   | Ser |     |         |       |
| 8 8 0 |     |     |      |       |     | 8 8 5 |       |     |     |       | 8 9 0 |       |       |       |     |     |         |       |
| GAC   | CTC | AAC | AAC  | CCG   | CGG | CTG   | CTG   | TAC | CCG | GGG   | GAC   | GTC   | AAC   | GGG   | TAC |     | 9 6 0   |       |
| Asp   | Leu | Asn | Asn  | Pro   | Arg | Leu   | Leu   | Tyr | Pro | Arg   | Asp   | Val   | Asn   | Gly   | Tyr |     |         |       |
| 8 9 5 |     |     |      |       |     | 9 0 0 |       |     |     |       | 9 0 5 |       |       |       |     |     |         |       |
| GGG   | CTG | GAA | GGG  | CAG   | TGG | AGC   | GAC   | GAC | TTC | CAC   | CAC   | GCC   | GTC   | CAC   | GTC |     | 1 0 0 8 |       |
| Gly   | Leu | Glu | Gly  | Gln   | Trp | Ser   | Asp   | Asp | Phe | His   | His   | Ala   | Val   | His   | Val |     |         |       |
| 9 1 0 |     |     |      | 9 1 5 |     |       |       |     |     | 9 2 0 |       |       |       | 9 2 5 |     |     |         |       |
| AAC   | GTC | ACC | GGC  | GAA   | ACC | ACC   | GGC   | TAC | TAC | AGT   | GAC   | TTC   | GAC   | TCG   | CTG |     | 1 0 5 6 |       |
| Asn   | Val | Thr | Gly  | Glu   | Thr | Thr   | Gly   | Tyr | Tyr | Ser   | Asp   | Phe   | Asp   | Ser   | Leu |     |         |       |
| 9 3 0 |     |     |      |       |     | 9 3 5 |       |     |     |       | 9 4 0 |       |       |       |     |     |         |       |

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|     |     |     |     |      |     |     |     |      |      |      |      |     |      |      |      |     |      |
|-----|-----|-----|-----|------|-----|-----|-----|------|------|------|------|-----|------|------|------|-----|------|
| GCC | GCC | CTC | GCC | AAG  | GTG | CTC | CGG | GAC  | GGC  | TTC  | TTC  | CAC | GAC  | GGC  | AGC  |     | 1104 |
| Ala | Ala | Leu | Ala | Lys  | Val | Leu | Arg | Asp  | Gly  | Phe  | Phe  | His | Asp  | Gly  | Ser  |     |      |
| 945 |     |     |     |      |     | 950 |     |      |      |      |      | 955 |      |      |      |     |      |
| TAC | TCC | AGC | TTC | CGG  | GAA | CGC | CAC | CAC  | GGA  | CGG  | CCG  | ATT | AAT  | TTC  | AGC  |     | 1152 |
| Tyr | Ser | Ser | Phe | Arg  | Glu | Arg | His | His  | Gly  | Arg  | Pro  | Ile | Asn  | Phe  | Ser  |     |      |
| 960 |     |     |     |      |     | 965 |     |      |      |      |      | 970 |      |      |      |     |      |
| GCC | GTA | CAC | CCA | GCC  | GCC | CTG | GTG | GTC  | TGT  | TCG  | CAG  | AAC | CAC  | GAC  | CAG  |     | 1200 |
| Ala | Val | His | Pro | Ala  | Ala | Leu | Val | Val  | Cys  | Ser  | Gln  | Asn | His  | Asp  | Gln  |     |      |
| 975 |     |     |     |      |     | 980 |     |      |      | 985  |      |     |      |      |      |     |      |
| ATC | GGC | AAC | CGT | GCC  | ACG | GGG | GAC | CGG  | CTC  | TCC  | CAG  | ACC | CTG  | CCG  | TAC  |     | 1248 |
| Ile | Gly | Asn | Arg | Ala  | Thr | Gly | Asp | Arg  | Leu  | Ser  | Gln  | Thr | Leu  | Pro  | Tyr  |     |      |
| 990 |     |     |     |      |     | 995 |     |      |      | 1000 |      |     |      |      | 1005 |     |      |
| GGA | AGC | CTG | GCC | CTC  | GCT | GCG | GTG | CTG  | ACC  | CTG  | ACG  | GGA | CCC  | TTC  | ACG  |     | 1296 |
| Gly | Ser | Leu | Ala | Leu  | Ala | Ala | Val | Leu  | Thr  | Leu  | Thr  | Gly | Pro  | Phe  | Thr  |     |      |
|     |     |     |     | 1010 |     |     |     |      | 1015 |      |      |     |      | 1020 |      |     |      |
| CCC | ATG | CTG | CTC | ATG  | GGC | GAG | GAG | TAC  | GGC  | GCC  | AGC  | ACG | CCG  | TGG  | CAG  |     | 1344 |
| Pro | Met | Leu | Leu | Met  | Gly | Glu | Glu | Tyr  | Gly  | Ala  | Ser  | Thr | Pro  | Trp  | Gln  |     |      |
|     |     |     |     | 1025 |     |     |     | 1030 |      |      |      |     | 1035 |      |      |     |      |
| TTT | TTC | ACC | TCG | CAC  | CCG | GAG | CCG | GAG  | CTC  | GGC  | AAG  | GCC | ACC  | GCG  | GAG  |     | 1392 |
| Phe | Phe | Thr | Ser | His  | Pro | Glu | Pro | Glut | Leu  | Gly  | Lys  | Ala | Thr  | Ala  | Glu  |     |      |
|     |     |     |     | 1040 |     |     |     | 1045 |      |      | 1050 |     |      |      |      |     |      |
| GGC | CGG | ATC | AAG | GAG  | TTC | GAG | CGC | ATG  | GGG  | TGG  | GAT  | CCC | GCC  | GTC  | GTG  |     | 1440 |
| Gly | Arg | Ile | Lys | Glu  | Phe | Phe | Arg | Met  | Gly  | Trp  | Asp  | Pro | Ala  | Val  | Val  |     |      |
|     |     |     |     | 1055 |     |     |     | 1060 |      | 1065 |      |     |      |      |      |     |      |
| CCC | GAT | CCC | CAG | GAT  | CCT | GAG | ACG | TTC  | CGC  | CGG  | TCC  | AAG | CTG  | GAC  | TGG  |     | 1488 |
| Pro | Asp | Pro | Gln | Asp  | Pro | Glu | Thr | Phe  | Arg  | Arg  | Ser  | Lys | Leu  | Asp  | Trp  |     |      |
|     |     |     |     | 1070 |     |     |     | 1075 |      | 1080 |      |     |      |      | 1085 |     |      |
| GCG | GAA | GCC | GCC | GAA  | GGC | GAC | CAT | GCC  | CGG  | CTG  | CTG  | GAG | CTG  | TAC  | CGT  |     | 1536 |
| Ala | Glu | Ala | Ala | Ala  | Glu | Gly | Asp | His  | Ala  | Arg  | Leu  | Leu | Glu  | Leu  | Tyr  | Arg |      |
|     |     |     |     | 1090 |     |     |     |      | 1095 |      |      |     |      | 1100 |      |     |      |
| TCG | CTC | ACC | GCC | CTG  | CGC | CGC | TCC | ACG  | CCG  | GAC  | CTC  | ACC | AAG  | CTG  | GGC  |     | 1584 |
| Ser | Leu | Thr | Ala | Leu  | Arg | Arg | Ser | Thr  | Pro  | Asp  | Leu  | Thr | Lys  | Leu  | Gly  |     |      |
|     |     |     |     | 1105 |     |     |     | 1110 |      |      |      |     |      | 1115 |      |     |      |
| TTC | GAG | GAC | ACG | CAG  | GTG | GCG | TTC | GAC  | GAG  | GAC  | GCC  | CGC | TGG  | CTG  | CGG  |     | 1632 |
| Phe | Glu | Asp | Thr | Gln  | Val | Ala | Phe | Asp  | Glu  | Asp  | Ala  | Arg | Trp  | Leu  | Arg  |     |      |
|     |     |     |     | 1120 |     |     |     | 1125 |      |      | 1130 |     |      |      |      |     |      |
| TTC | CGC | CGG | GGT | GGC  | GTG | CAG | GTG | CTG  | CTC  | AAC  | TTC  | TCG | GAA  | CAG  | CCC  |     | 1680 |
| Phe | Arg | Arg | Gly | Gly  | Val | Gln | Val | Leu  | Leu  | Asn  | Phe  | Ser | Glu  | Gln  | Pro  |     |      |
|     |     |     |     | 1135 |     |     |     | 1140 |      | 1145 |      |     |      |      |      |     |      |
| GTG | AGC | CTG | GAC | GGG  | GCG | GGC | ACG | GCC  | CTG  | CTG  | GCC  | ACC | GAC  | GAC  |      |     | 1728 |
| Val | Ser | Leu | Asp | Gly  | Ala | Gly | Thr | Ala  | Leu  | Leu  | Leu  | Ala | Thr  | Asp  | Asp  |     |      |
|     |     |     |     | 1150 |     |     |     | 1155 |      |      | 1160 |     |      |      | 1165 |     |      |
| GCC | GTC | CGG | CTA | GAA  | GGT | GAG | CGT | GCG  | GAA  | CTC  | GGT  | CCG | CTG  | AGC  | GCC  |     | 1776 |
| Ala | Val | Arg | Leu | Glu  | Gly | Glu | Arg | Ala  | Glu  | Leu  | Gly  | Pro | Leu  | Ser  | Ala  |     |      |
|     |     |     |     | 1170 |     |     |     |      | 1175 |      |      |     |      | 1180 |      |     |      |
| GCC | GTC | GTC | AGC | GAC  |     |     |     |      |      |      |      |     |      |      |      |     | 1791 |
| Ala | Val | Val | Ser | Asp  |     |     |     |      |      |      |      |     |      |      |      |     |      |
|     |     |     |     | 1185 |     |     |     |      |      |      |      |     |      |      |      |     |      |

## ( 2 ) INFORMATION FOR SEQ ID NO:4:

## ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 597 amino acids
- ( B ) TYPE: amino acid
- ( D ) TOPOLOGY: linear

## ( i i ) MOLECULE TYPE: protein

## ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Thr His Thr Tyr Pro Arg Glu Ala Ala Lys Pro Val Leu Gly Pro Ala
1      5

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Arg Tyr Asp Val Trp Ala Pro Asn Ala Glu Ser Val Thr Leu Leu Ala

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| 2 0   | 2 5 | 3 0 |
|---|-----|-----|
| G l y   G l y   G l u   A r g   T y r   A l a   M e t   G l n   A r g   A r g   A l a   G l u   T h r   G l y   P r o   G l u<br>3 5                  4 0                  4 5                              |     |     |
| A s p   A l a   G l y   T r p   T r p   T h r   A l a   A l a   G l y   A l a   P r o   T h r   A s p   G l y   A s n   V a l<br>5 0                  5 5                  6 0                  6 5         |     |     |
| A s p   T y r   G l y   T y r   L e u   L e u   A s p   G l y   A s p   G l u   T h r   P r o   L e u   P r o   A s p   P r o<br>6 5                  7 0                  7 5                  8 0         |     |     |
| A r g   T h r   A r g   A r g   G l n   P r o   A s p   G l y   V a l   H i s   A l a   L e u   S e r   A r g   T h r   P h e<br>8 5                  9 0                  9 5                              |     |     |
| A s p   P r o   S e r   A l a   T y r   S e r   T r p   G l n   A s p   A s p   A l a   T r p   G l n   G l y   A r g   G l u<br>1 0 0  |     |     |
| L e u   G l n   G l y   A l a   V a l   I l e   T y r   G l u   L e u   H i s   L e u   G l y   T h r   P h e   T h r   P r o<br>1 1 5                  1 2 0                  1 2 5                        |     |     |
| G l u   G l y   T h r   L e u   G l u   A l a   A l a   A l a   G l y   L y s   L e u   A s p   T y r   L e u   A l a   G l y<br>1 3 0                  1 3 5   |     |     |
| L e u   G l y   V a l   A s p   P h e   I l e   G l u   L e u   L e u   P r o   V a l   A s n   A l a   P h e   A s n   G l y<br>1 4 5                  1 5 0                  1 5 5                  1 6 0 |     |     |
| T h r   H i s   A s n   T r p   G l y   T y r   A s p   G l y   V a l   G l n   T r p   P h e   A l a   V a l   H i s   G l u<br>1 6 5                  1 7 0                  1 7 5                        |     |     |
| A l a   T y r   G l y   G l y   P r o   G l u   A l a   T y r   G l n   A r g   P h e   V a l   A s p   A l a   A l a   H i s<br>1 8 0                  1 8 5                  1 9 0                        |     |     |
| A l a   A l a   G l y   L e u   G l y   V a l   I l e   G l n   A s p   V a l   V a l   T y r   A s n   H i s   L e u   G l y<br>1 9 5                  2 0 0                  2 0 5                        |     |     |
| P r o   S e r   G l y   A s n   T y r   L e u   P r o   A r g   P h e   G l y   P r o   T y r   L e u   L y s   G l n   G l y<br>2 1 0                  2 1 5   |     |     |
| G l u   G l y   A s n   T h r   T r p   G l y   A s p   S e r   V a l   A s n   L e u   A s p   G l y   P r o   G l y   S e r<br>2 2 5                  2 3 0                  2 3 5                  2 4 0 |     |     |
| A s p   H i s   V a l   A r g   A r g   T y r   I l e   L e u   A s p   A s n   L e u   A l a   M e t   T r p   L e u   A r g<br>2 4 5                  2 5 0                  2 5 5                        |     |     |
| A s p   T y r   A r g   V a l   A s p   G l y   L e u   A r g   L e u   A s p   A l a   V a l   H i s   A l a   L e u   L y s<br>2 6 0                  2 6 5                  2 7 0                        |     |     |
| A s p   G l u   A r g   A l a   V a l   H i s   I l e   L e u   G l u   A s p   P h e   G l y   A l a   L e u   A l a   A s p<br>2 7 5                  2 8 0                  2 8 5                        |     |     |
| G l n   I l e   S e r   A l a   G l u   V a l   G l y   A r g   P r o   L e u   T h r   L e u   I l e   A l a   G l u   S e r<br>2 9 0                  2 9 5                  3 0 0                        |     |     |
| A s p   L e u   A s n   A s n   P r o   A r g   L e u   L e u   T y r   P r o   A r g   A s p   V a l   A s n   G l y   T y r<br>3 0 5                  3 1 0                  3 1 5                  3 2 0 |     |     |
| G l y   L e u   G l u   G l y   G l n   T r p   S e r   A s p   A s p   P h e   H i s   H i s   A l a   V a l   H i s   V a l<br>3 2 5                  3 3 0                  3 3 5                        |     |     |
| A s n   V a l   T h r   G l y   G l u   T h r   T h r   G l y   T y r   T y r   S e r   A s p   P h e   A s p   S e r   L e u<br>3 4 0                  3 4 5                  3 5 0                        |     |     |
| A l a   A l a   L e u   A l a   L y s   V a l   L e u   A r g   A s p   G l y   P h e   P h e   H i s   A s p   G l y   S e r<br>3 5 5                  3 6 0                  3 6 5                        |     |     |
| T y r   S e r   S e r   P h e   A r g   G l u   A r g   H i s   H i s   G l y   A r g   P r o   I l e   A s n   P h e   S e r<br>3 7 0                  3 7 5                  3 8 0                        |     |     |
| A l a   V a l   H i s   P r o   A l a   A l a   L e u   V a l   V a l   C y s   S e r   G l n   A s n   H i s   A s p   G l n<br>3 8 5                  3 9 0                  3 9 5                        |     |     |
| I l e   G l y   A s n   A r g   A l a   T h r   G l y   A s p   A r g   L e u   S e r   G l n   T h r   L e u   P r o   T y r<br>4 0 5                  4 1 0                  4 1 5                        |     |     |
| G l y   S e r   L e u   A l a   L e u   A l a   A l a   V a l   L e u   T h r   L e u   T h r   G l y   P r o   P h e   T h r<br>4 2 0                  4 2 5                  4 3 0                        |     |     |
| P r o   M e t   L e u   L e u   M e t   G l y   G l u   G l u   T y r   G l y   A l a   S e r   T h r   P r o   T r p   G l n<br>4 3 5                  4 4 0                  4 4 5                        |     |     |

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Phe | Thr | Ser | His | Pro | Glu | Pro | Glu | Leu | Gly | Lys | Ala | Thr | Ala | Glu |
| 450 |     |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Gly | Arg | Ile | Lys | Glu | Phe | Glu | Arg | Met | Gly | Trp | Asp | Pro | Ala | Val | Val |
| 465 |     |     |     |     | 470 |     |     |     | 475 |     |     |     |     |     | 480 |
| Pro | Asp | Pro | Gln | Asp | Pro | Glu | Thr | Phe | Arg | Arg | Ser | Lys | Leu | Asp | Trp |
| 485 |     |     |     |     |     |     |     | 490 |     |     |     |     |     | 495 |     |
| Ala | Glu | Ala | Ala | Glu | Gly | Asp | His | Ala | Arg | Leu | Leu | Glu | Leu | Tyr | Arg |
|     | 500 |     |     |     |     |     |     | 505 |     |     |     |     |     |     |     |
| Ser | Leu | Thr | Ala | Leu | Arg | Arg | Ser | Thr | Pro | Asp | Leu | Thr | Lys | Leu | Gly |
|     | 515 |     |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |
| Phe | Glu | Asp | Thr | Gln | Val | Ala | Phe | Asp | Glu | Asp | Ala | Arg | Trp | Leu | Arg |
| 530 |     |     |     |     | 535 |     |     |     |     |     | 540 |     |     |     |     |
| Phe | Arg | Arg | Gly | Gly | Val | Gln | Val | Leu | Leu | Asn | Phe | Ser | Glu | Gln | Pro |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
| Val | Ser | Leu | Asp | Gly | Ala | Gly | Thr | Ala | Leu | Leu | Leu | Ala | Thr | Asp | Asp |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |
| Ala | Val | Arg | Leu | Glu | Gly | Glu | Arg | Ala | Glu | Leu | Gly | Pro | Leu | Ser | Ala |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     |     |     |     |
| Ala | Val | Val | Ser | Asp |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 595 |     |     |     |     |     |     |     |     |     |     |     |     |

( 2 ) INFORMATION FOR SEQ ID NO:5:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 20 amino acids
- ( B ) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: peptide

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:5:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Lys | Pro | Val | Gln | Gly | Ala | Gly | Arg | Phe | Asp | Ile | Trp | Ala | Pro | Glu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     |     | 15  |
| Ala | Gly | Thr | Val |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

( 2 ) INFORMATION FOR SEQ ID NO:6:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 20 amino acids
- ( B ) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: peptide

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:6:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | His | Thr | Tyr | Pro | Arg | Glu | Ala | Ala | Lys | Pro | Val | Leu | Gly | Pro | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     |     | 15  |
| Arg | Tyr | Asp | Val |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

( 2 ) INFORMATION FOR SEQ ID NO:7:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 21 amino acids
- ( B ) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: peptide

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5,830,715

39

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|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| P r o | V a l | G l n | G l y | A l a | G l y | A r g | P h e | A s p | I l e | T r p | A l a | P r o | G l u | A l a | G l y |
| 1     |       |       | 5     |       |       |       |       |       | 1 0   |       |       |       |       | 1 5   |       |
| T h r | V a l | T h r | L e u | L e u |       |       |       |       |       |       |       |       |       |       |       |
|       |       |       |       |       | 2 0   |       |       |       |       |       |       |       |       |       |       |

( 2 ) INFORMATION FOR SEQ ID NO:8:

- ( i ) SEQUENCE CHARACTERISTICS:  
( A ) LENGTH: 17 amino acids  
( B ) TYPE: amino acid  
( C ) STRANDEDNESS: single  
( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: peptide

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:8:

|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| L e u | A s p | T r p | A l a | G l u | A l a | S e r | A l a | G l y | A s p | H i s | A l a | A r g | L e u | L e u | G l u |
| 1     |       |       |       | 5     |       |       |       |       | 1 0   |       |       |       |       | 1 5   |       |
| L e u |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |

( 2 ) INFORMATION FOR SEQ ID NO:9:

- ( i ) SEQUENCE CHARACTERISTICS:  
( A ) LENGTH: 20 amino acids  
( B ) TYPE: amino acid  
( C ) STRANDEDNESS: single  
( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: peptide

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:9:

|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| G l u | P h e | G l u | A r g | M e t | G l y | T r p | A s p | P r o | A l a | V a l | V a l | P r o | A s p | P r o | G l n |
| 1     |       |       |       | 5     |       |       |       |       | 1 0   |       |       |       |       | 1 5   |       |
| A s p | P r o | G l u | T h r |       |       |       |       |       |       |       |       |       |       |       |       |
|       |       |       |       | 2 0   |       |       |       |       |       |       |       |       |       |       |       |

( 2 ) INFORMATION FOR SEQ ID NO:10:

- ( i ) SEQUENCE CHARACTERISTICS:  
( A ) LENGTH: 20 amino acids  
( B ) TYPE: amino acid  
( C ) STRANDEDNESS: single  
( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: peptide

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:10:

|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| P r o | V a l | L e u | G l y | P r o | A l a | A r g | T y r | A s p | V a l | T r p | A l a | P r o | A s n | A l a | G l u |
| 1     |       |       | 5     |       |       |       |       |       | 1 0   |       |       |       |       | 1 5   |       |
| S e r | V a l | T h r | L e u |       |       |       |       |       |       |       |       |       |       |       |       |
|       |       |       |       | 2 0   |       |       |       |       |       |       |       |       |       |       |       |

( 2 ) INFORMATION FOR SEQ ID NO:11:

- ( i ) SEQUENCE CHARACTERISTICS:  
( A ) LENGTH: 2161 base pairs  
( B ) TYPE: nucleic acid  
( C ) STRANDEDNESS: single  
( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( i x ) FEATURE:

- ( A ) NAME/KEY: CDS  
( B ) LOCATION: 207..1994

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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| 8 8 0  |       | 8 8 5 |       | 8 9 0   |       |         |
|--|-------|-------|-------|---------|-------|---------|
| A C C  | C T C | A T C | G C C | G A G   | T C C | G A C   |
| Thr  | Leu   | Ile   | Ala   | Gl u    | Ser   | A sp    |
| 8 9 5  |       |       |       | 9 0 0   |       |         |
| C G G  | G A T | G T C | A A C | G G C   | T A C | G G A   |
| Arg  | Asp   | Val   | Asn   | Gly     | Tyr   | Gly     |
|  |       |       |       | 9 1 5   |       |         |
| C A C  | C A C | G C C | G T G | C A C   | G T C | A A C   |
| His  | His   | Ala   | Val   | His     | Val   | Asn     |
|  |       |       |       | 9 3 0   |       |         |
| A G C  | G A C | T T C | G A C | T C G   | C T C | G G A   |
| Ser  | Asp   | Phe   | Asp   | Ser     | Leu   | Gly     |
|  |       |       |       | 9 4 5   |       |         |
| T T C  | T T C | C A C | G A C | G G C   | T A C | G A C   |
| Phe  | Phe   | His   | Asp   | Gly     | Ser   | Tyr     |
|  |       |       |       | 9 6 0   |       |         |
| C G G  | C C G | A T C | A A C | T T C   | A G C | G G G   |
| Arg  | Pro   | Ile   | Asn   | Phe     | Ser   | Gly     |
|  |       |       |       | 9 7 5   |       |         |
| T C A  | C A G | A A C | C A T | G A C   | C A G | A A C   |
| Ser  | Gln   | Asn   | His   | Asp     | Gln   | Ile     |
|  |       |       |       | 9 9 5   |       |         |
| T C C  | C A G | T C A | C T T | C C G   | T A C | G G C   |
| Ser  | Gln   | Ser   | Leu   | Pro     | Tyr   | Gly     |
|  |       |       |       | 1 0 1 0 |       |         |
| C T C  | A C C | G G T | C C G | T T C   | A C G | C C C   |
| Leu  | Thr   | Gly   | Pro   | Phe     | Thr   | Pro     |
|  |       |       |       | 1 0 2 5 |       |         |
| G C C  | A C C | A C C | C C G | T G G   | C A G | T T C   |
| Ala  | Thr   | Thr   | Pro   | Trp     | Gln   | Phe     |
|  |       |       |       | 1 0 4 0 |       |         |
| G G C  | A A G | G C C | A C G | G C C   | G A G | G G G   |
| Gly  | Lys   | Ala   | Thr   | Ala     | Glu   | Gly     |
|  |       |       |       | 1 0 5 5 |       |         |
| T G G  | G A T | C C C | G C C | G T C   | G T G | T T C   |
| Trp  | Asp   | Pro   | Ala   | Val     | Val   | Pro     |
|  |       |       |       | 1 0 7 5 |       |         |
| C G C  | T C C | A A A | C T G | G A C   | T G G | C G G   |
| Arg  | Ser   | Lys   | Leu   | Asp     | Trp   | Ala     |
|  |       |       |       | 1 0 9 0 |       |         |
| C T C  | C T G | G A G | C T G | T A C   | C G C | T C G   |
| Leu  | Leu   | Glu   | Leu   | Tyr     | Arg   | Ser     |
|  |       |       |       | 1 1 0 5 |       |         |
| G A G  | C T C | G C G | C G C | C T G   | G G C | T T C   |
| Glu  | Leu   | Ala   | Arg   | Leu     | Gly   | Phe     |
|  |       |       |       | 1 1 2 0 |       |         |
| G A C  | G C C | C G C | T G G | C T C   | C G T | T A G   |
| Asp  | Ala   | Arg   | Trp   | Leu     | Arg   | Tyr     |
|  |       |       |       | 1 1 3 5 |       |         |
| A A C  | T T C | G C G | G A C | C G T   | T A T | T G G   |
| Asn  | Phe   | Ala   | Asp   | Arg     | Pro   | Trp     |
|  |       |       |       | 1 1 5 5 |       |         |
| C T G  | C T C | G C C | A C C | G A C   | G C C | G G A   |
| Leu  | Leu   | Ala   | Thr   | Asp     | Ala   | Val     |
|  |       |       |       | 1 1 7 0 |       |         |
| C T G  | C C G | C T G | A G C | G C G   | G T G | G A G   |
| Leu  | Pro   | Pro   | Leu   | Ser     | Ala   | Ala     |
|  |       |       |       | 1 1 8 5 |       |         |
| G C G G G C G T C C    T T C C G G T G A C    C G G A T G C T G G    A C G C C C G C C C    C G C A G C T C C A    C A G G C G C T G G G |       |       |       |         |       |         |
|  |       |       |       |         |       | 2 0 7 4 |

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|  |      |
|--|------|
| CAGGATGGAA CGTATGACTT TTCTGGCAGC GGACAAACCGC TACGAAACCA TGCCATACCG | 2134 |
| CCGCCTCGGA CGCAGCGGGC TGAAGCT                                      | 2161 |

( 2 ) INFORMATION FOR SEQ ID NO:12:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 596 amino acids
- ( B ) TYPE: amino acid
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Thr Gln Pro Asn Asp Ala Ala Lys Pro Val Gln Gly Ala Gly Arg
 1           5           10          15

Phe Asp Ile Trp Ala Pro Glu Ala Gly Thr Val Thr Leu Leu Ala Gly
20          25          30

Gly Glu Arg Tyr Glu Met Gly Arg Arg Pro Gly Asn Gly Pro Ala Asp
35          40          45          50          55          60

Glu Gly Trp Trp Thr Ala Ala Asp Ala Pro Thr Gly Ala Asp Val Asp
50          55          60          65          70          75          80

Tyr Gly Tyr Leu Leu Asp Gly Asp Glu Ile Pro Leu Pro Asp Pro Arg
65          70          75          80          85          90          95

Thr Arg Arg Gln Pro Glu Gly Val His Ala Leu Ser Arg Thr Phe Asp
85          90          95          100          105          110          115

Pro Gly Ala His Arg Trp Gln Asp Ala Gly Trp Gln Gly Arg Glu Leu
100          105          110          115          120          125          130

Gln Gly Ser Val Ile Tyr Glu Leu His Ile Gly Thr Phe Thr Pro Glu
115          120          125          130          135          140          145

Gly Thr Leu Asp Ala Ala Ala Gly Lys Leu Asp Tyr Leu Ala Gly Leu
130          135          140          145          150          155          160

Gly Ile Asp Phe Ile Glu Leu Leu Pro Val Asn Ala Phe Asn Gly Thr
145          150          155          160          165          170          175

His Asn Trp Gly Tyr Asp Gly Val Gln Trp Phe Ala Val His Glu Gly
165          170          175          180          185          190          195

Tyr Gly Gly Pro Ala Ala Tyr Gln Arg Phe Val Asp Ala Ala His Ala
180          185          190          195          200          205          210

Ala Gly Leu Gly Val Ile Gln Asp Val Val Tyr Asn His Leu Gly Pro
195          200          205          210          215          220          225

Ser Gly Asn Tyr Leu Pro Arg Tyr Gly Pro Tyr Leu Lys His Gly Glu
210          215          220          225          230          235          240

Gly Asn Thr Trp Gly Asp Ser Val Asn Leu Asp Gly Pro Gly Ser Asp
225          230          235          240          245          250          255

His Val Arg Gln Tyr Ile Leu Asp Asn Val Ala Met Trp Leu Arg Asp
245          250          255          260          265          270          275

Tyr Arg Val Asp Gly Leu Arg Leu Asp Ala Val His Ala Leu Lys Asp
260          265          270          275          280          285          290

Glu Arg Ala Val His Ile Leu Glu Glu Phe Gly Ala Leu Ala Asp Ala
275          280          285          290          295          300          305

Leu Ser Ser Glu Gly Gly Arg Pro Leu Thr Leu Ile Ala Glu Ser Asp
290          295          300          305          310          315          320

Leu Asn Asn Pro Arg Leu Leu Tyr Pro Arg Asp Val Asn Gly Tyr Gly
305          310          315          320          325          330          335

Leu Ala Gly Gln Trp Ser Asp Asp Phe His His Ala Val His Val Asn
325          330          335          340          345          350          355

Val Ser Gly Glu Thr Thr Gly Tyr Tyr Ser Asp Phe Asp Ser Leu Gly
340          345          350          355          360          365          370

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|       | 3 4 0 | 3 4 5 | 3 5 0 |
|-------|-------|-------|-------|
| Ala   | Leu   | Ala   | Lys   |
| 3 5 5 | Val   | Leu   | Arg   |
|       |       | Asp   | Gly   |
|       |       | 3 6 0 | Phe   |
| Ser   | Ser   | Phe   | Arg   |
| 3 7 0 | Gly   | Arg   | Cys   |
|       | 3 7 5 | His   | Gly   |
|       |       | Arg   | Pro   |
|       |       | 3 8 0 | Ile   |
| Val   | His   | Pro   | Ala   |
| 3 8 5 | Ala   | Ala   | Leu   |
|       | Val   | Val   | Cys   |
|       |       | Ser   | Gln   |
|       |       | 3 9 5 | Asn   |
|       |       |       | His   |
|       |       |       | Asp   |
|       |       |       | Gln   |
|       |       |       | 4 0 0 |
| Gly   | Asn   | Arg   | Ala   |
| 4 0 5 | Thr   | Gly   | Asp   |
|       |       | Arg   | Leu   |
|       |       | Ser   | Gln   |
|       |       | 4 1 0 | Ser   |
| Ser   | Leu   | Ala   | Leu   |
| 4 2 0 | Ala   | Ala   | Val   |
|       |       | Leu   | Thr   |
|       |       | 4 2 5 | Leu   |
|       |       |       | Thr   |
|       |       |       | Gly   |
|       |       |       | Pro   |
|       |       |       | Phe   |
|       |       |       | Thr   |
|       |       |       | Pro   |
| Met   | Leu   | Phe   | Met   |
| 4 3 5 | Gly   | Glu   | Glu   |
|       |       | Tyr   | Gly   |
|       | 4 4 0 | Ala   | Thr   |
|       |       | Thr   | Thr   |
|       |       | 4 4 5 | Pro   |
| Phe   | Thr   | Ser   | His   |
| 4 5 0 | Pro   | Glu   | Pro   |
|       |       | Glut  | Glu   |
|       | 4 5 5 | Leu   | Gly   |
|       |       | Lys   | Ala   |
|       |       | 4 6 0 | Thr   |
|       |       |       | Ala   |
|       |       |       | Glu   |
|       |       |       | Gly   |
| Arg   | Ile   | Arg   | Glu   |
| 4 6 5 | Phe   | Glu   | Phe   |
|       |       | Arg   | Met   |
|       | 4 7 0 | Gly   | Gly   |
|       |       | Trp   | Asp   |
|       |       | 4 7 5 | Pro   |
|       |       |       | Ala   |
|       |       |       | Val   |
|       |       |       | Val   |
|       |       |       | Pro   |
| Asp   | Pro   | Gln   | Asp   |
| 4 8 5 | Pro   | Glu   | Thr   |
|       |       | Phe   | Thr   |
|       |       | 4 9 0 | Arg   |
|       |       |       | Ser   |
|       |       |       | Lys   |
|       |       |       | Leu   |
|       |       |       | Asp   |
|       |       |       | Trp   |
|       |       |       | Ala   |
| Glu   | Ala   | Ser   | Ala   |
| 5 0 0 | Gly   | Asp   | His   |
|       |       | Ala   | Arg   |
|       |       | 5 0 5 | Leu   |
|       |       |       | Leu   |
|       |       |       | Glu   |
|       |       |       | Leu   |
|       |       |       | Tyr   |
|       |       | 5 1 0 | Arg   |
|       |       |       | Ser   |
| Leu   | Ile   | Thr   | Leu   |
| 5 1 5 | Arg   | Arg   | Ser   |
|       |       | 5 2 0 | Thr   |
|       |       |       | Pro   |
|       |       |       | Glu   |
|       |       |       | Leu   |
|       |       |       | Ala   |
|       |       |       | Arg   |
|       |       | 5 2 5 | Leu   |
|       |       |       | Gly   |
|       |       |       | Phe   |
| Ala   | Asp   | Thr   | Ala   |
| 5 3 0 | Val   | Glu   | Phe   |
|       |       |       | Asp   |
|       | 5 3 5 |       | Asp   |
|       |       |       | Asp   |
|       |       |       | Ala   |
|       |       |       | Arg   |
|       |       | 5 4 0 | Trp   |
|       |       |       | Leu   |
|       |       |       | Arg   |
|       |       |       | Tyr   |
| Trp   | Arg   | Gly   | Gly   |
| 5 4 5 | Val   | Gln   | Val   |
|       |       |       | Val   |
|       | 5 5 0 | Leu   | Asn   |
|       |       |       | Phe   |
|       |       | 5 5 5 | Ala   |
|       |       |       | Asp   |
|       |       |       | Arg   |
|       |       |       | Phe   |
|       |       |       | Ile   |
| Ser   | Leu   | Asp   | Arg   |
| 5 6 5 | Pro   | Gly   | Thr   |
|       |       | Ala   | Leu   |
|       |       | 5 7 0 | Leu   |
|       |       |       | Ala   |
|       |       |       | Thr   |
|       |       |       | Asp   |
|       |       | 5 7 5 | Asp   |
|       |       |       | Ala   |
| Val   | Arg   | Met   | Asp   |
| 5 8 0 | Gly   | Val   | Gln   |
|       |       |       | Val   |
|       |       | 5 8 5 | Glu   |
|       |       |       | Leu   |
|       |       |       | Pro   |
|       |       |       | Pro   |
|       |       |       | Leu   |
|       |       |       | Ser   |
|       |       | 5 9 0 | Ala   |
|       |       |       | Ala   |
|       |       |       | Ala   |
| Val   | Leu   | Arg   | Asp   |
| 5 9 5 |       |       |       |

## ( 2 ) INFORMATION FOR SEQ ID NO:13:

## ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 2056 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

## ( i i ) MOLECULE TYPE: cDNA

## ( i x ) FEATURE:

- ( A ) NAME/KEY: CDS
- ( B ) LOCATION: 90..1883

## ( xi ) SEQUENCE DESCRIPTION: SEQ ID NO:13:

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| GCCGGCTTCG | GACCGGGGGC | AGTGAAGATC | GCCGACATCT | TCCGGTCGTT | CCCCGTTGCG | 6 0 |
| CTGCTGGTGC | CGCAGACAGG | AGGAGAGTC  | ATG        | ACG        | CAC        | ACC |
|            |            |            | Met        | Thr        | His        | Thr |
|            |            |            |            |            | Tyr        | Tyr |
|            |            |            |            |            | Pro        | Pro |
|            |            |            |            |            | Arg        | Arg |
|            |            |            |            |            | Glu        | Glu |
| GCC        | GCG        | AAA        | CCC        | GTC        | CTG        | GGC |
| Ala        | Ala        | Lys        | Pro        | Val        | Leu        | Gly |
| 6 0 5      |            |            |            | 6 1 0      | Pro        | Ala |
|            |            |            |            |            | Arg        | Arg |
|            |            |            |            |            | Tyr        | Tyr |
|            |            |            |            |            | Asp        | Asp |
|            |            |            |            |            | Val        | Val |
|            |            |            |            |            | Trp        | Trp |
|            |            |            |            |            | Ala        | Ala |
|            |            |            |            |            | Pro        | Pro |
| AAC        | GCT        | GAA        | TCC        | GTC        | ACG        | CTG |
| Asn        | Ala        | Glu        | Ser        | Val        | Thr        | Leu |
|            |            |            |            | 6 2 5      |            | Ala |
|            |            |            |            |            |            | Gly |
|            |            |            |            |            |            | Gly |
|            |            |            |            |            |            | Gly |
|            |            |            |            |            |            | Glu |
|            |            |            |            |            |            | Arg |
|            |            |            |            |            |            | Tyr |
|            |            |            |            |            |            | Ala |
|            |            |            |            |            |            | Met |
|            |            |            |            |            | 6 3 5      |     |
|            |            |            |            |            |            |     |

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|     |     |     |       |     |       |       |       |       |       |     |       |       |       |     |     |         |
|-----|-----|-----|-------|-----|-------|-------|-------|-------|-------|-----|-------|-------|-------|-----|-----|---------|
| CAG | CGC | CGG | GCC   | GAG | ACC   | GGG   | CCG   | GAG   | GAC   | GCC | GGC   | TGG   | TGG   | ACC | GCC | 2 5 7   |
| Gln | Arg | Arg | Ala   | Glu | Thr   | Gly   | Pro   | Glu   | Asp   | Ala | Gly   | Trp   | Trp   | Thr | Ala |         |
|     |     |     | 6 4 0 |     |       |       | 6 4 5 |       |       |     |       | 6 5 0 |       |     |     |         |
| GCC | GGC | GCG | CCT   | ACG | GAT   | GGC   | AAC   | GTG   | GAC   | TAC | GGG   | TAC   | CTT   | CTG | GAC | 3 0 5   |
| Ala | Gly | Ala | Pro   | Thr | Asp   | Gly   | Asn   | Val   | Asp   | Tyr | Gly   | Tyr   | Leu   | Leu | Asp |         |
|     |     |     | 6 5 5 |     |       |       | 6 6 0 |       |       |     |       | 6 6 5 |       |     |     |         |
| GGC | GAC | GAA | ACA   | CCG | CTT   | CCG   | GAT   | CCA   | CGG   | ACC | CGC   | CGC   | CAG   | CCC | GAC | 3 5 3   |
| Gly | Asp | Glu | Thr   | Pro | Leu   | Pro   | Asp   | Pro   | Arg   | Thr | Arg   | Arg   | Gln   | Pro | Asp |         |
|     |     |     | 6 7 0 |     |       | 6 7 5 |       |       |       |     | 6 8 0 |       |       |     |     |         |
| GGC | GTC | CAC | GCC   | CTG | TCC   | CGC   | ACG   | TTC   | GAC   | CCG | TCC   | GCG   | TAC   | AGC | TGG | 4 0 1   |
| Gly | Val | His | Ala   | Leu | Ser   | Arg   | Thr   | Phe   | Asp   | Pro | Ser   | Ala   | Tyr   | Ser | Trp |         |
|     |     |     | 6 8 5 |     | 6 9 0 |       |       | 6 9 5 |       |     |       |       | 7 0 0 |     |     |         |
| CAG | GAC | GAC | GCC   | TGG | CAG   | GGC   | AGG   | GAA   | CTG   | CAG | GGC   | GCC   | GTC   | ATC | TAC | 4 4 9   |
| Gln | Asp | Asp | Ala   | Trp | Gln   | Gly   | Arg   | Glu   | Leu   | Gln | Gly   | Ala   | Val   | Ile | Tyr |         |
|     |     |     | 7 0 5 |     |       |       |       | 7 1 0 |       |     |       |       | 7 1 5 |     |     |         |
| GAG | CTC | CAC | CTC   | GGA | ACA   | TTC   | ACG   | CCC   | GAA   | GGG | ACG   | CTG   | GAG   | GCG | GCC | 4 9 7   |
| Glu | Leu | His | Leu   | Gly | Thr   | Phe   | Thr   | Pro   | Glu   | Gly | Thr   | Leu   | Glu   | Ala | Ala |         |
|     |     |     | 7 2 0 |     |       |       | 7 2 5 |       |       |     |       | 7 3 0 |       |     |     |         |
| GCC | GGA | AAG | CTG   | GAC | TAC   | CTC   | GCC   | GGC   | TTG   | GGC | GTC   | GAC   | TTC   | ATC | GAG | 5 4 5   |
| Ala | Gly | Lys | Leu   | Asp | Tyr   | Leu   | Ala   | Gly   | Leu   | Gly | Val   | Asp   | Phe   | Ile | Glu |         |
|     |     |     | 7 3 5 |     |       | 7 4 0 |       |       | 7 4 5 |     |       |       |       |     |     |         |
| CTG | CTG | CCG | GTC   | AAC | GCT   | TTC   | AAC   | GCC   | GTC   | GAC | AAC   | TGG   | GGT   | TAC | GAC | 5 9 3   |
| Leu | Leu | Pro | Val   | Asn | Ala   | Phe   | Asn   | Gly   | Leu   | Gly | Asn   | Trp   | Gly   | Tyr | Asp |         |
|     |     |     | 7 5 0 |     |       | 7 5 5 |       |       | 7 6 0 |     |       |       |       |     |     |         |
| GGT | GTC | CAG | TGG   | TTC | GCT   | GTG   | CAC   | GAG   | GCA   | TAC | GGC   | GGG   | CCG   | GAA | GCG | 6 4 1   |
| Gly | Val | Gln | Trp   | Phe | Ala   | Val   | His   | Glu   | Ala   | Tyr | Gly   | Gly   | Pro   | Glu | Ala |         |
|     |     |     | 7 6 5 |     | 7 7 0 |       |       | 7 7 5 |       |     |       |       | 7 8 0 |     |     |         |
| TAC | CAG | CGG | TTC   | GTC | GAC   | GCC   | GCC   | CAC   | GCC   | GCA | GGC   | CTT   | GGC   | GTG | ATC | 6 8 9   |
| Tyr | Gln | Arg | Phe   | Val | Asp   | Ala   | Ala   | His   | Ala   | Ala | Gly   | Leu   | Gly   | Val | Ile |         |
|     |     |     | 7 8 5 |     |       |       | 7 9 0 |       |       |     |       | 7 9 5 |       |     |     |         |
| CAG | GAC | GTG | GTC   | TAC | AAC   | CAC   | CTC   | GGC   | CCC   | AGC | GGG   | AAC   | TAC   | CTG | CCG | 7 3 7   |
| Gln | Asp | Val | Val   | Tyr | Asn   | His   | Leu   | Gly   | Pro   | Ser | Gly   | Asn   | Tyr   | Leu | Pro |         |
|     |     |     | 8 0 0 |     |       |       | 8 0 5 |       |       |     |       | 8 1 0 |       |     |     |         |
| CGG | TTC | GGG | CCG   | TAC | CTC   | AAG   | CAG   | GCG   | GAG   | GGT | AAC   | ACG   | TGG   | GGC | GAC | 7 8 5   |
| Arg | Phe | Gly | Pro   | Tyr | Leu   | Lys   | Gln   | Gly   | Glu   | Gly | Asn   | Thr   | Trp   | Gly | Asp |         |
|     |     |     | 8 1 5 |     |       | 8 2 0 |       |       | 8 2 5 |     |       |       |       |     |     |         |
| TCG | GTG | AAC | CTG   | GAC | GGG   | CCC   | GCC   | GCG   | TCC   | GAC | CAT   | GTG   | CGC   | CGG | TAC | 8 3 3   |
| Ser | Val | Asn | Leu   | Asp | Gly   | Pro   | Gly   | Gly   | Ser   | Asp | His   | Val   | Arg   | Arg | Tyr |         |
|     |     |     | 8 3 0 |     |       | 8 3 5 |       |       |       |     | 8 4 0 |       |       |     |     |         |
| CTG | GAC | AAC | CTG   | GCC | ATG   | TGG   | CTG   | CGT   | GAC   | TAC | CGG   | GTG   | GAC   | GGC | CTG | 8 8 1   |
| Leu | Asp | Asn | Leu   | Ala | Met   | Trp   | Leu   | Arg   | Asp   | Tyr | Arg   | Val   | Asp   | Gly | Leu |         |
|     |     |     | 8 4 5 |     | 8 5 0 |       |       | 8 5 5 |       |     |       |       | 8 6 0 |     |     |         |
| CGG | CTG | GAC | GCC   | GTC | CAC   | GCC   | CTG   | AAG   | GAT   | GAG | CGG   | GTG   | CAC   | ATC | ATC | 9 2 9   |
| Arg | Leu | Asp | Ala   | Val | His   | Ala   | Leu   | Lys   | Asp   | Glu | Arg   | Ala   | Val   | Ile | Ile |         |
|     |     |     | 8 6 5 |     |       |       | 8 7 0 |       |       |     |       | 8 7 5 |       |     |     |         |
| CTG | GAG | GAC | TTC   | GGG | GCG   | CTG   | GCC   | GAT   | CAG   | ATC | TCC   | GCC   | GAG   | GTG | GGA | 9 7 7   |
| Leu | Glu | Asp | Phe   | Gly | Ala   | Leu   | Ala   | Asp   | Gln   | Ile | Ser   | Ala   | Glu   | Val | Gly |         |
|     |     |     | 8 8 0 |     |       | 8 8 5 |       |       | 8 9 0 |     |       |       |       |     |     |         |
| CGG | CCG | CTG | ACG   | CTC | ATC   | GCC   | GAG   | TCC   | GAC   | CTC | AA    | AAC   | CCG   | CGG | CTG | 1 0 2 5 |
| Arg | Pro | Leu | Thr   | Leu | Ile   | Ala   | Glu   | Ser   | Asp   | Leu | Asn   | Asn   | Pro   | Arg | Leu |         |
|     |     |     | 8 9 5 |     |       | 9 0 0 |       |       | 9 0 5 |     |       |       |       |     |     |         |
| CTG | TAC | CCG | CGG   | GAC | GTC   | AAC   | GGG   | TAC   | GGG   | CTG | GAA   | GGG   | CAG   | TGG | AGC | 1 0 7 3 |
| Leu | Tyr | Pro | Arg   | Asp | Val   | Asn   | Gly   | Tyr   | Gly   | Leu | Glu   | Gly   | Gln   | Trp | Ser |         |
|     |     |     | 9 1 0 |     |       | 9 1 5 |       |       | 9 2 0 |     |       |       |       |     |     |         |
| GAC | GAC | TTC | CAC   | CAC | GCC   | GTC   | CAC   | GTC   | AAC   | GTC | ACC   | GGC   | GAA   | ACC | ACC | 1 1 2 1 |
| Asp | Asp | Phe | His   | His | Ala   | Val   | His   | Val   | Asn   | Val | Thr   | Gly   | Glu   | Thr | Thr |         |
|     |     |     | 9 2 5 |     | 9 3 0 |       |       | 9 3 5 |       |     |       |       | 9 4 0 |     |     |         |
| GGC | TAC | TAC | TAC   | AGT | GAC   | TTC   | GAC   | TCG   | CTG   | GCC | CTC   | GCC   | AAG   | GTG | CTC | 1 1 6 9 |
| Gly | Tyr | Tyr | Tyr   | Ser | Asp   | Phe   | Asp   | Ser   | Leu   | Ala | Leu   | Ala   | Lys   | Val | Leu |         |
|     |     |     | 9 4 5 |     |       |       |       |       | 9 5 0 |     |       |       | 9 5 5 |     |     |         |

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|                     |                     |                     |                     |                     |                     |       |       |       |       |         |       |         |       |       |       |     |         |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-------|-------|-------|-------|---------|-------|---------|-------|-------|-------|-----|---------|
| C G G               | G A C               | G G C               | T T C               | T T C               | C A C               | G A C | G G C | A G C | T A C | T C C   | A G C | T T C   | C G G | G A A | C G C |     | 1 2 1 7 |
| Arg                 | Asp                 | Gly                 | Phe                 | Phe                 | His                 | Asp   | Gly   | Ser   | Tyr   | Ser     | Ser   | Phe     | Arg   | Glu   | Arg   |     | 9 6 0   |
| 9 6 5               |                     |                     |                     |                     |                     |       |       |       |       |         |       |         | 9 7 0 |       |       |     |         |
| C A C               | C A C               | G G A               | C G G               | C C G               | A T T               | A A T | T T C | A G C | G C C | G T A   | C A C | C C A   | G C C | G C C | C T G |     | 1 2 6 5 |
| His                 | His                 | Gly                 | Arg                 | Pro                 | Ile                 | Asn   | Phe   | Ser   | Ala   | Val     | His   | Pro     | Ala   | Ala   | Leu   |     | 9 7 5   |
| 9 8 0               |                     |                     |                     |                     |                     |       |       |       |       |         |       | 9 8 5   |       |       |       |     |         |
| G T G               | G T C               | T G T               | T C G               | C A G               | A A C               | C A C | G A C | C A G | A T C | G G C   | A A C | C G T   | G C C | A C G | G G G |     | 1 3 1 3 |
| Val                 | Val                 | Cys                 | Ser                 | Gln                 | Asn                 | His   | Asp   | Gln   | Ile   | Gly     | Asn   | Arg     | Ala   | Thr   | Gly   |     | 9 9 0   |
| 9 9 5               |                     |                     |                     |                     |                     |       |       |       |       | 1 0 0 0 |       |         |       |       |       |     |         |
| G A C               | C G G               | C T C               | T C C               | C A G               | A C C               | C T G | C C G | T A C | G G A | A G C   | C T G | G C C   | C T C | G C T | G C G |     | 1 3 6 1 |
| Asp                 | Arg                 | Leu                 | Ser                 | Gln                 | Thr                 | Leu   | Pro   | Tyr   | Gly   | Ser     | Leu   | Ala     | Leu   | Ala   | Ala   |     | 1 0 0 5 |
| 1 0 1 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 0 1 5 |       |         |       |       |       |     |         |
| G T G               | C T G               | A C C               | C T G               | A C G               | G G A               | C C C | T T C | A C G | C C C | A T G   | C T G | C T C   | A T G | G G C | G A G |     | 1 4 0 9 |
| Val                 | Leu                 | Thr                 | Leu                 | Thr                 | Gly                 | Pro   | Phe   | Thr   | Pro   | Met     | Leu   | Leu     | Met   | Gly   | Glu   |     | 1 0 2 5 |
| 1 0 3 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 0 3 5 |       |         |       |       |       |     |         |
| G A G               | T A C               | G G C               | G C C               | A G C               | A C G               | C C G | T G G | C A G | T T T | T T C   | A C C | T C G   | C A C | C C G | G A G |     | 1 4 5 7 |
| Glu                 | Tyr                 | Gly                 | Ala                 | Ser                 | Thr                 | Pro   | Trp   | Gln   | Phe   | Phe     | Thr   | Ser     | His   | Pro   | Glu   |     | 1 0 4 0 |
| 1 0 4 5             |                     |                     |                     |                     |                     |       |       |       |       |         |       | 1 0 5 0 |       |       |       |     |         |
| C C G               | G A G               | C T C               | G G C               | A A G               | G C C               | A C C | G C G | G A G | G G C | C G G   | A T C | A A G   | G A G | T T C | G A G |     | 1 5 0 5 |
| Pro                 | Glu                 | Leu                 | Gly                 | Lys                 | Ala                 | Thr   | Ala   | Glu   | Gly   | Arg     | Ile   | Lys     | Glu   | Phe   | Glu   |     | 1 0 5 5 |
| 1 0 6 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 0 6 5 |       |         |       |       |       |     |         |
| C G C               | A T G               | G G G               | T G G               | G A T               | C C C               | G C C | G T C | G T G | C C C | G A T   | C C C | C A G   | G A T | C C T | G A G |     | 1 5 5 3 |
| Arg                 | Met                 | Gly                 | Trp                 | Asp                 | Pro                 | Ala   | Val   | Val   | Pro   | Asp     | Pro   | Gln     | Asp   | Pro   | Glu   |     | 1 0 7 0 |
| 1 0 7 5             |                     |                     |                     |                     |                     |       |       |       |       | 1 0 8 0 |       |         |       |       |       |     |         |
| A C G               | T T C               | C G C               | C G G               | T C C               | A A G               | C T G | G A C | T G G | G C G | G A A   | G C C | G C C   | G A A | G G C | G A C |     | 1 6 0 1 |
| Thr                 | Phe                 | Arg                 | Arg                 | Ser                 | Lys                 | Leu   | Asp   | Trp   | Ala   | Glu     | Ala   | Ala     | Ala   | Glu   | Gly   | Asp | 1 0 8 5 |
| 1 0 9 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 0 9 5 |       |         |       |       |       |     |         |
| C A T               | G C C               | C G G               | C T G               | C T G               | G A G               | C T G | T A C | C G T | T C G | C T C   | A C C | G C C   | C T G | C G C | C G C |     | 1 6 4 9 |
| His                 | Ala                 | Arg                 | Leu                 | Leu                 | Glu                 | Leu   | Tyr   | Arg   | Ser   | Leu     | Thr   | Ala     | Leu   | Arg   | Arg   |     | 1 1 0 5 |
| 1 1 1 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 1 1 5 |       |         |       |       |       |     |         |
| T C C               | A C G               | C C G               | G A C               | C T C               | A C C               | A A G | C T G | G G C | T T C | G A G   | G A C | A C G   | C A G | G T G | G C G |     | 1 6 9 7 |
| Ser                 | Thr                 | Pro                 | Asp                 | Leu                 | Thr                 | Lys   | Leu   | Gly   | Phe   | Glu     | Asp   | Thr     | Gln   | Val   | Ala   |     | 1 1 2 0 |
| 1 1 2 5             |                     |                     |                     |                     |                     |       |       |       |       | 1 1 3 0 |       |         |       |       |       |     |         |
| T T C               | G A C               | G A G               | G A C               | G C C               | C G C               | T G G | C T G | C G G | T T C | C G C   | C G G | G G T   | G G C | G T G | C A G |     | 1 7 4 5 |
| Phe                 | Asp                 | Glu                 | Asp                 | Ala                 | Arg                 | Trp   | Leu   | Arg   | Phe   | Arg     | Arg   | Gly     | Gly   | Va l  | Gln   |     | 1 1 3 5 |
| 1 1 4 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 1 4 5 |       |         |       |       |       |     |         |
| G T G               | C T G               | C T C               | A A C               | T T C               | T C G               | G A A | C A G | C C C | G T G | A G C   | C T G | G A C   | G G G | G C G | G G C |     | 1 7 9 3 |
| Val                 | Leu                 | Leu                 | Asn                 | Phe                 | Ser                 | Glu   | Gln   | Pro   | Val   | Ser     | Leu   | Asp     | Gly   | Ala   | Gly   |     | 1 1 5 0 |
| 1 1 5 5             |                     |                     |                     |                     |                     |       |       |       |       | 1 1 6 0 |       |         |       |       |       |     |         |
| A C G               | G C C               | C T G               | C T G               | C T G               | G C C               | A C C | G A C | G A C | G C C | G T C   | C G G | C T A   | G A A | G G T | G A G |     | 1 8 4 1 |
| Thr                 | Ala                 | Leu                 | Leu                 | Leu                 | Leu                 | Ala   | Thr   | Asp   | Ala   | Val     | Arg   | Leu     | Glu   | Gly   | Glu   |     | 1 1 6 5 |
| 1 1 7 0             |                     |                     |                     |                     |                     |       |       |       |       | 1 1 7 5 |       |         |       |       |       |     |         |
| C G T               | G C G               | G A A               | C T C               | G G T               | C C G               | C T G | A G C | G C C | G C C | G T C   | G T C | A G C   | G A C |       |       |     | 1 8 8 3 |
| Arg                 | Ala                 | Glu                 | Leu                 | Gly                 | Pro                 | Leu   | Ser   | Ala   | Ala   | Val     | Val   | Ser     | Asp   |       |       |     | 1 1 8 5 |
| 1 1 9 0             |                     |                     |                     |                     |                     |       |       |       |       |         |       |         |       |       |       |     |         |
| T G A C G T T T T C | T T G G G G G C G G | C G T C C A C C G C | C G G T G A C C G G | A T G G T G G A C G | T C C G C C C C G A |       |       |       |       |         |       |         |       |       |       |     | 1 9 4 3 |
| A G C C T C G G C G | C G G C T G G C A G | G A T G G A A C G C | A T G A C T T A T G | T G G C C T C G G A | C A C C C G C T A C |       |       |       |       |         |       |         |       |       |       |     | 2 0 0 3 |
| G A C A C C A T G C | C C T A C C G C C G | C G T C G G A C G C | A G C G G C C T C A | A A C T G C C G G C | C A T               |       |       |       |       |         |       |         |       |       |       |     | 2 0 5 6 |

( 2 ) INFORMATION FOR SEQ ID NO:14:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 598 amino acids
- ( B ) TYPE: amino acid
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Thr His Thr Tyr Pro Arg Glu Ala Ala Lys Pro Val Leu Gly Pro

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| 1     | 5     | 10    | 15    |
|-------|-------|-------|-------|
| A l a | A r g | T y r | A s p |
| 2 0   | V a l | T r p | A l a |
|       |       |       | P r o |
|       |       |       | A s n |
|       |       |       | A l a |
|       |       |       | G l u |
|       |       |       | S e r |
|       |       |       | V a l |
|       |       |       | T h r |
|       |       |       | L e u |
|       |       |       | L e u |
| A l a | G l y | G l y | G l u |
| 3 5   | G l u | A r g | T y r |
|       |       | A l a | M e t |
|       |       |       | G l n |
|       |       |       | A r g |
|       |       |       | A r g |
|       |       |       | A l a |
|       |       |       | G l u |
|       |       |       | T h r |
|       |       |       | G l y |
|       |       |       | P r o |
| G l u | A s p | A l a | G l y |
| 5 0   | T r p | T r p | T h r |
|       |       |       | A l a |
|       |       |       | A l a |
|       |       |       | G l y |
|       |       |       | A l a |
|       |       |       | P r o |
|       |       |       | T h r |
|       |       |       | A s p |
|       |       |       | G l y |
|       |       |       | A s n |
| V a l | A s p | T y r | G l y |
| 6 5   | T y r | L e u | L e u |
|       |       |       | A s p |
|       |       |       | G l y |
|       |       |       | A s p |
|       |       |       | G l u |
|       |       |       | T h r |
|       |       |       | P r o |
|       |       |       | L e u |
|       |       |       | P r o |
|       |       |       | A s p |
| P r o | A r g | T h r | A r g |
| 8 5   | A r g | G l n | P r o |
|       |       |       | A s p |
|       |       |       | G l y |
|       |       |       | V a l |
|       |       |       | H i s |
|       |       |       | A l a |
|       |       |       | L e u |
|       |       |       | S e r |
|       |       |       | A r g |
|       |       |       | T h r |
| P h e | A s p | P r o | S e r |
| 1 0 0 | A l a | T y r | S e r |
|       |       |       | T r p |
|       |       |       | G l n |
|       |       |       | A s p |
|       |       |       | A s p |
|       |       |       | A l a |
|       |       |       | T r p |
|       |       |       | G l n |
|       |       |       | G l y |
|       |       |       | A r g |
| G l u | L e u | G l n | G l y |
| 1 1 5 | A l a | V a l | I l e |
|       |       |       | T y r |
|       |       |       | G l u |
|       |       |       | L e u |
|       |       |       | H i s |
|       |       |       | L e u |
|       |       |       | G l y |
|       |       |       | T h r |
|       |       |       | P h e |
|       |       |       | T h r |
| P r o | G l u | G l y | T h r |
| 1 3 0 | L e u | G l u | A l a |
|       |       |       | A l a |
|       |       |       | A l a |
|       |       |       | G l y |
|       |       |       | L y s |
|       |       |       | L e u |
|       |       |       | A s p |
|       |       |       | T y r |
|       |       |       | L e u |
|       |       |       | A l a |
| G l y | T h r | H i s | A s n |
| 1 6 5 | A s n | T r p | G l y |
|       |       |       | T y r |
|       |       |       | A s p |
|       |       |       | G l y |
|       |       |       | V a l |
|       |       |       | G l n |
|       |       |       | T r p |
|       |       |       | P h e |
|       |       |       | A l a |
|       |       |       | V a l |
|       |       |       | H i s |
| G l u | A l a | T y r | G l y |
| 1 8 0 | G l y | P r o | G l u |
|       |       |       | A l a |
|       |       |       | T y r |
|       |       |       | G l n |
|       |       |       | A r g |
|       |       |       | P h e |
|       |       |       | V a l |
|       |       |       | A s p |
|       |       |       | A l a |
|       |       |       | A l a |
| H i s | A l a | A l a | G l y |
| 1 9 5 | L e u | G l y | V a l |
|       |       |       | I l e |
|       |       |       | G l n |
|       |       |       | A s p |
|       |       |       | V a l |
|       |       |       | V a l |
|       |       |       | T y r |
|       |       |       | A s n |
|       |       |       | H i s |
|       |       |       | L e u |
| G l y | P r o | S e r | G l y |
| 2 1 0 | A s n | T y r | A s n |
|       |       |       | L e u |
|       |       |       | P r o |
|       |       |       | A r g |
|       |       |       | T y r |
|       |       |       | P h e |
|       |       |       | G l y |
|       |       |       | P r o |
|       |       |       | T y r |
|       |       |       | L e u |
|       |       |       | L y s |
|       |       |       | G l n |
| G l y | G l u | G l y | A s n |
| 2 2 5 | T h r | T r p | G l y |
|       |       |       | A s p |
|       |       |       | S e r |
|       |       |       | V a l |
|       |       |       | A s n |
|       |       |       | L e u |
|       |       |       | A l a |
|       |       |       | M e t |
|       |       |       | T r p |
|       |       |       | L e u |
| A r g | A s p | T y r | A r g |
| 2 6 0 | V a l | A s p | G l y |
|       |       |       | L e u |
|       |       |       | A r g |
|       |       |       | L e u |
|       |       |       | A s p |
|       |       |       | A l a |
|       |       |       | V a l |
|       |       |       | H i s |
|       |       |       | A l a |
|       |       |       | L e u |
| L y s | A s p | G l u | A r g |
| 2 7 5 | A l a | V a l | H i s |
|       |       |       | I l e |
|       |       |       | L e u |
|       |       |       | G l u |
|       |       |       | A s p |
|       |       |       | P h e |
|       |       |       | G l y |
|       |       |       | A l a |
|       |       |       | L e u |
|       |       |       | A l a |
| A s p | G l n | I l e | S e r |
| 2 9 0 | S e r | A l a | G l u |
|       |       |       | V a l |
|       |       |       | G l y |
|       |       |       | A r g |
|       |       |       | T y r |
|       |       |       | P h e |
|       |       |       | G l y |
|       |       |       | P r o |
|       |       |       | G l y |
| S e r | A s p | L e u | A s n |
| 3 0 5 | A s n | A s n | P r o |
|       |       |       | A r g |
|       |       |       | L e u |
|       |       |       | L e u |
|       |       |       | T y r |
|       |       |       | P r o |
|       |       |       | A r g |
|       |       |       | T y r |
|       |       |       | H i s |
|       |       |       | A l a |
|       |       |       | V a l |
|       |       |       | H i s |
| T y r | G l y | L e u | G l u |
| 3 2 5 | G l y | G l n | T r p |
|       |       |       | S e r |
|       |       |       | A s p |
|       |       |       | A s p |
|       |       |       | P h e |
|       |       |       | H i s |
|       |       |       | H i s |
|       |       |       | A l a |
|       |       |       | V a l |
|       |       |       | H i s |
| V a l | A s n | V a l | T h r |
| 3 4 0 | G l y | G l u | T h r |
|       |       |       | T h r |
|       |       |       | G l y |
|       |       |       | T y r |
|       |       |       | T y r |
|       |       |       | S e r |
|       |       |       | A s p |
|       |       |       | P h e |
|       |       |       | V a l |
|       |       |       | H i s |
|       |       |       | A s p |
|       |       |       | G l y |
| L e u | A l a | A l a | L e u |
| 3 5 5 | A l a | A l a | L y s |
|       |       |       | V a l |
|       |       |       | L e u |
|       |       |       | A r g |
|       |       |       | A s p |
|       |       |       | G l y |
|       |       |       | P h e |
|       |       |       | P h e |
| S e r | T y r | S e r | S e r |
| 3 7 0 | S e r | P h e | A r g |
|       |       |       | G l u |
|       |       |       | A r g |
|       |       |       | T y r |
|       |       |       | H i s |
|       |       |       | P r o |
|       |       |       | A s n |
|       |       |       | P h e |
| S e r | A l a | V a l | H i s |
| 3 8 5 | P r o | A l a | A l a |
|       |       |       | L e u |
|       |       |       | V a l |
|       |       |       | V a l |
|       |       |       | C y s |
|       |       |       | S e r |
|       |       |       | G l n |
|       |       |       | A s n |
|       |       |       | H i s |
|       |       |       | A s p |
| G l n | I l e | G l y | A s n |
| 4 0 5 | A s n | A r g | A l a |
|       |       |       | T h r |
|       |       |       | G l y |
|       |       |       | A s p |
|       |       |       | G l y |
| T y r | G l y | S e r | L e u |
| 4 2 0 | A l a | L e u | A l a |
|       |       |       | A l a |
|       |       |       | V a l |
|       |       |       | 4 2 5 |

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**55**

**56**

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Met | Leu | Leu | Met | Gly | Glu | Glu | Tyr | Gly | Ala | Ser | Thr | Pro | Trp |
| 435 |     |     |     |     |     | 440 |     |     |     | 445 |     |     |     |     |     |
| Gln | Phe | Phe | Thr | Ser | His | Pro | Glu | Pro | Glu | Leu | Gly | Lys | Ala | Thr | Ala |
| 450 |     |     |     |     |     | 455 |     |     |     | 460 |     |     |     |     |     |
| Glu | Gly | Arg | Ile | Lys | Glu | Phe | Glu | Arg | Met | Gly | Trp | Asp | Pro | Ala | Val |
| 465 |     |     |     |     | 470 |     |     |     | 475 |     |     |     |     |     | 480 |
| Val | Pro | Asp | Pro | Gln | Asp | Pro | Glu | Thr | Phe | Arg | Arg | Ser | Lys | Leu | Asp |
|     |     |     |     | 485 |     |     |     | 490 |     |     |     |     | 495 |     |     |
| Trp | Ala | Glu | Ala | Ala | Glu | Gly | Asp | His | Ala | Arg | Leu | Leu | Glu | Leu | Tyr |
|     |     |     |     | 500 |     |     |     | 505 |     |     |     |     | 510 |     |     |
| Arg | Ser | Leu | Thr | Ala | Leu | Arg | Arg | Ser | Thr | Pro | Asp | Leu | Thr | Lys | Leu |
|     |     |     |     |     |     | 520 |     |     |     |     |     | 525 |     |     |     |
| Gly | Phe | Glu | Asp | Thr | Gln | Val | Ala | Phe | Asp | Glu | Asp | Ala | Arg | Trp | Leu |
|     |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |     |
| Arg | Phe | Arg | Arg | Gly | Gly | Val | Gln | Val | Leu | Leu | Asn | Phe | Ser | Glu | Gln |
|     |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
| Pro | Val | Ser | Leu | Asp | Gly | Ala | Gly | Thr | Ala | Leu | Leu | Leu | Ala | Thr | Asp |
|     |     |     |     | 565 |     |     |     | 570 |     |     |     |     | 575 |     |     |
| Asp | Ala | Val | Arg | Leu | Glu | Gly | Glu | Arg | Ala | Glu | Leu | Gly | Pro | Leu | Ser |
|     |     |     |     | 580 |     |     |     | 585 |     |     |     |     | 590 |     |     |
| Ala | Ala | Val | Val | Ser | Asp |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 595 |     |     |     |     |     |     |     |     |     |     |     |

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( 2 ) INFORMATION FOR SEQ ID NO:15:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 17 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:15:

T T Y G A Y A T H T    G G G C N C C

1 7

( 2 ) INFORMATION FOR SEQ ID NO:16:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 17 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:16:

G T A A A A C G A C    G G C C A G T

1 7

( 2 ) INFORMATION FOR SEQ ID NO:17:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 17 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:17:

A T G G G N T G G G    A Y C C N G C

1 7

( 2 ) INFORMATION FOR SEQ ID NO:18:

-continued

( i ) SEQUENCE CHARACTERISTICS:  
 ( A ) LENGTH: 14 base pairs  
 ( B ) TYPE: nucleic acid  
 ( C ) STRANDEDNESS: single  
 ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:18:

T A Y G A Y G T N T    G G G C

1 4

We claim:

1. A method for converting a non-reducing saccharide, comprising the step of:

introducing a recombinant DNA carrying a self-replicable vector and a nucleotide sequence coding for the expression of a variant of an enzyme of SEQ ID NO: 2 or SEQ ID NO: 4, which variant acts on a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher to release trehalose, into a host microorganism to obtain a recombinant microorganism, wherein the variant has an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 with one or more amino acid residues in SEQ ID NO: 2 or SEQ ID NO: 4 deleted, replaced with different amino acids, or supplemented with one or more amino acids without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4;

culturing said recombinant microorganism having said recombinant DNA for expression of said variant enzyme in a nutrient culture medium to accumulate said variant enzyme;

recovering said accumulated variant enzyme; and

subjecting said non-reducing saccharide to the action of said recovered variant enzyme possessing trehalose releasing activity to convert said non-reducing saccharide.

2. The method according to claim 1, wherein said non-reducing saccharide is prepared by successively treating an

<sup>15</sup> amylaceous substance selected from the group consisting of starch, amylopectin, amylose, and mixtures thereof, with acid in combination with or without amylase.

3. The method according to claim 1, wherein said non-reducing saccharide is selected from the group consisting of <sup>20</sup>  $\alpha$ -glucosyltrehalose,  $\alpha$ -maltosyltrehalose,  $\alpha$ -maltotriosyltrehalose,  $\alpha$ -maltotetraosyltrehalose,  $\alpha$ -maltopentaoosyltrehalose, and mixtures thereof.

4. The method according to claim 1, wherein said non-reducing saccharide has a concentration of 50 w/v % or lower in solution, and said subjecting step is carried out at a temperature of about 40°–55° C. and a pH in a range of about 6–8.

5. The method according to claim 1, wherein the variant has one or more amino acid residues in SEQ ID NO: 2 or <sup>30</sup> SEQ ID NO: 4 replaced with different amino acids without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4.

6. The method according to claim 1, wherein the variant has one or more amino acid residues in SEQ ID NO: 2 or <sup>35</sup> SEQ ID NO: 4 deleted without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4.

7. The method according to claim 1, wherein the variant has one or more amino acid residues in SEQ ID NO: 2 or <sup>40</sup> SEQ ID NO: 4 supplemented with one or more amino acids without substantially altering the activity of the enzyme of SEQ ID NO: 2 or SEQ ID NO: 4.

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